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COMPARATIVE VIGOR OF F_1 WHEAT CROSSES AND THEIR PARENTS [1]

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The comparative vigor of F_1 crosses and their parents is a subject of much interest to the plant breeder. In crops where the technic of crossing is comparatively easy, the increase in vigor obtained in the F_1 cross often more than pays for the additional trouble of producing the hybrid seed. In self-fertilized crops like the small grains where considerable labor is involved in making artificial crosses, it is apparent that F_1 crosses can not be used commercially as a means of increasing crop yields. The suggestion, however, has been made by Anderson (*1*)[3] that the added vigor of the heterozygous condition might be utilized in small grains by making a large number of crosses between strains which, when crossed, show a considerable increase in yield. Produce from F_2 and F_3 progeny could be used for seeding the general field, and the crosses could be repeated each year in order to keep up the supply of seed.

Several theories have been advanced to explain the phenomenon of heterosis. The discovery of genetic linkage has led to the development of an adequate Mendelian explanation of the vigor so often obtained in F_1 crosses. An excellent review of the development of this theory is given by East and Jones (*8*). The theory explains the increase in vigor shown in the first hybrid generation as being due to the meeting in the zygote of dominant or partially dominant growth factors some of which are contributed by each parent. Linkage is given as the reason why all dominant factors can not be combined in a homozygous individual. According to this hypothesis the maximum number of favorable growth factors can be obtained only in the heterozygous condition.

In producing new varieties by crossing, forms may be obtained in the F_3 generation which appear homozygous for botanical and agronomic char-

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[3] Reference is made by number (italic) to "Literature cited," p. 62–63.

acters but which may be heterozygous for growth factors. There is the possibility that this heterozygous condition may cause the F_2 or F. hybrid to give a high yield. After several further generations this heterozygous condition may be lost, with a consequent loss in growth stimulus. A knowledge of the amount of added vigor in the F_1 generation is of value in determining whether heterozygosis in F_2 and F_4 lines would modify their yields sufficiently to interfere seriously with a determination of their value as improved varieties.

With these points in view a study has been made in wheat of the immediate effect of cross-pollination on seed weight and the increased vigor of F_1 crosses. Pure lines were used of seven varieties of *Triticum vulgare* Vill. and one variety of each *T. compactum* Host. (Little Club), *T. dicoccum* Schr. (Spring Emmer), and *T. durum* Desf. (Mindum). Varieties of *T. vulgare* were crossed with each other and with Little Club, Spring Emmer, and Mindum. Little Club was crossed also with Spring Emmer and Mindum.

IMMEDIATE EFFECT OF CROSS-POLLINATION

Because of the phenomenon of double fertilization it is possible in some cases to obtain an increase in weight of seed as an immediate effect of cross-pollination. The increase is due principally to an increase in weight of endosperm in such crops as corn, where the proportion of endosperm to embryo is large.

Collins (*5*) observed open-pollinated ears of Chinese maize in which the size of seed was increased by cross-pollination. Seeds which showed by their color the effect of foreign pollen averaged 0.178 gm., while white seeds from the same portion of the ear averaged 0.153 gm. Roberts (*14*) mentions a similar instance with Chinese maize. Collins and Kempton (*6*) compared the average seed weight of corn from intravarietal and intervarietal pollinations. The intervarietal crosses exceeded the intravarietal in seed weight by 8.8 per cent. In a similar experiment, Wolfe (*16*) found that 23 of 31 corn varietal crosses yielded more grain than intravarietal pollinations. Carrier (*4*) obtained an increase in yield of grain in strains of corn when grown in a mixture as compared with any one of the strains grown alone.

That an increase is also obtained in the size of the embryo is clearly shown by Lewis and Vincent (*12*) in a comparison of seeds of Newtown apple from self- and cross-pollinations. The crossed seeds showed a striking increase in weight over that of the selfed seeds. As there is little or no endosperm in apple seeds, an increase in seed weight is due largely to an increased size of the cotyledons.

Since artificially pollinated seeds of wheat are usually smaller than normally pollinated seeds, spikes of each variety were emasculated in the same manner as for cross-pollination and then pollinated with pollen from plants of the same pure line. Seed from this intrapollination is

termed "incrossed seed" and is used as a basis of comparison in determining the immediate effect of cross-pollination. The average weight of a normally pollinated seed for all varieties used was 26.65 ± 0.22 mgm.,[1] and the average for an incrossed seeds was 18.13 ± 0.24 mgm.

A comparison is shown in Table I of the hybrid seed and the incrossed seed where the average dates of pollination are the same or approximately so.

TABLE I.—*Weight of seed of the immediate crosses compared with weight of seed of the incrossed parents*

Name of cross.	Seed parent.		Cross.		Difference between cross and female parent.
	Number of seeds.	Average weight of seeds.	Number of seeds.	Average weight of seeds.	
		Mgm.		*Mgm.*	*Mgm.*
Marquis × Velvet Chaff[a]	38	12.6±0.5	48	15.6±0.5	+3.0±0.7
Marquis × Penny	38	12.6± .5	24	20.2±1.0	+7.6±1.2
Haynes Bluestem × Marquis	49	17.2± .8	26	23.5± .7	+6.3±1.0
Little Club × Marquis	39	10.1± .5	50	9.4± .3	−0.7± .6
Emmer × Velvet Chaff	44	26.4± .8	24	27.1±1.3	+0.7±1.5
Velvet Chaff × Mindum	104	19.9± .6	23	15.9± .6	−4.0± .8
Emmer × Little Club	44	26.4± .8	15	25.0±1.2	−1.4±1.4

[a] In the discussion of crosses the seed parent is given first.

The varietal crosses in every case showed an increased seed weight as compared with the female parent. The largest increase in seed weight was 7.6 ± 1.2 mgm., which was obtained from the cross Marquis × Penny. This hybrid gave on the average over 50 per cent heavier seeds than incrossed Marquis. Of the species crosses none gave a significant increase in seed weight. Velvet Chaff crossed with Mindum produced seeds which on the average were 4.0 ± 0.8 mgm. lighter than the seeds of incrossed Velvet Chaff.

F_1 GENERATION CROSSES COMPARED WITH THEIR PARENTS

Some of the earliest hybridization work affords good examples of the vigor of F_1 crosses. For an excellent review of this subject the reader is referred to the publication of East and Jones (*8*).

In the present experiment the F_1 generations and their parents were grown in the greenhouse under controlled conditions. Care was taken to plant seeds at a uniform depth, and when the seedlings were about 4 inches tall they were transplanted to 7-inch pots, two seedlings to a pot and only like seedlings together. Unfortunately an epidemic of stem-rust

[1] The probable error of an average of averages was calculated according to the formula:

$$E = \frac{1}{N}\sqrt{n_1^2 e_1^2 + n_2^2 e_2^2 - - - - n_n^2 e_n^2},$$

in which n is the number of individuals in a generation, e the probable error, and N the total number of individuals (*12*).

started about heading time, and some plants were rusted badly Measurements of height were taken on those plants which were not attacked previous to heading, and yield data were taken only on plants uninjured by rust.

Both incrossed and normally pollinated seeds of the parental varieties were planted. Arny and Garber (*2*) have shown that in some cases there is a positive correlation between weight of seed planted and the vigor of resultant plants. In order to determine whether the size of seed planted was of importance in an analysis of individual plant yields in the present experiment, correlation coefficients were calculated for the weight of seed planted as subject and length of culm and yield of grain per plant as relative. (Table II.)

TABLE II.—*Correlation coefficients for weight of seed planted and the vigor of resultant plants*

Variety.	Coefficient of correlation with weight of seed planted as subject.		
	Length of tallest culm (relative).	Total culm length (relative).	Yield of grain in grams per plant (relative).
Marquis	−0.084±0.100	+0.136±0.098	+0.047±0.173
Velvet Chaff	+.069±.065	+.078±.065	+.087±.108
Barletta	−.144±.085	.205±.083	
Penny	−.197±.075	−.129±.077	+.010±.112
H. B. S. 1–16–12	−.242±.071	+.121±.071	−.194±.095
Bobs	−.116±.079	+.169±.078	+.205±.084
Little Club	−.118±.082	+.185±.080	+.032±.099
Emmer	+.024±.086	−.007±.085	−.425±.080
Mindum	−.121±.076	+.381±.066	+.136±.095

The only significant correlation was obtained with the Mindum variety. A correlation coefficient of +0.381±0.066 was obtained for weight of seed planted and total culm length. In the light of these facts it was considered legitimate to use the plants from normal and incrossed seed as a single parent population.

The F_1 crosses and their parents were compared for height of tallest culm and for total culm length. (Table III.)

Six of the 11 F_1 varietal crosses showed an increase in length of tallest culm as compared with the parental average, and 5 showed a decrease. The two F_1 crosses between Mindum and vulgare varieties were considerably taller than either parent. Similar results were obtained from crosses between Emmer and the same vulgare varieties. On the other hand, the F_1 crosses of Little Club with either Emmer or Mindum did not show a significant difference in average height of tallest culm when compared with the average of the parents.

In Table IV the crosses and their parents are compared for total culm length.

TABLE III.—*Height of tallest culm of F_1 wheat crosses compared with parental average*

Name of one parent.	Number of individuals.	Height.	Name of other parent.	Number of individuals.	Height.	Average height of parents.	F_1 cross. Number of individuals.	F_1 cross. Height.
		Inches.			*Inches.*	*Inches.*		*Inches.*
Marquis	46	46.5	Velvet chaff	105	44.7	45.6	64	42.3
	46	46.5	Barletta	60	48.7	47.6	38	50.8
	46	46.5	Penny	74	37.4	41.9	45	41.1
	46	46.5	Bobs	70	40.1	43.3	65	47.9
Velvet Chaff	105	44.7	Barletta	60	48.7	46.7	38	50.9
	105	44.7	Penny	74	37.4	41.0	49	42.6
	105	44.7	Bobs	70	40.1	42.4	108	40.7
Penny	74	37.4		70	40.1	38.7	60	33.6
Haynes Bluestem	79	50.8	Marquis	46	46.5	48.6	24	50.8
Marquis	46	46.5	Little Club	66	49.2	47.8	62	50.5
Velvet Chaff	105	44.7		66	49.2	46.9	52	43.3
Average	73	45.4		69	43.8	44.6	55	45.0
Emmer	62	51.9	Little Club	66	49.2	50.5	12	51.0
Mindum	77	49.7		66	49.2	49.4	2	48.5
	77	49.7	Marquis	46	46.5	48.1	14	53.2
	77	49.7	Velvet Chaff	105	44.7	47.2	17	54.5
Emmer	62	51.9		105	44.7	48.3	28	55.5
	62	51.9	Marquis	46	46.5	49.2	18	55.6
Average	70	50.8		72	46.8	48.8	15	53.1

TABLE IV.—*Total culm length of F_1 wheat crosses compared with parental average*

Name of one parent.	Number of individuals.	Height.	Name of other parent.	Number of individuals.	Height.	Average height of parents.	F_1 cross. Number of individuals.	F_1 cross. Height.
		Inches.			*Inches.*	*Inches.*		*Inches.*
Marquis	46	195	Velvet Chaff	105	149	172	64	146
	46	195	Barletta	60	157	176	38	168
	46	195	Penny	74	90	143	45	132
	46	195	Bobs	70	136	166	65	211
	46	195	Little Club	66	149	172	62	167
Velvet Chaff	105	149	Barletta	60	157	153	38	174
	105	149	Penny	74	90	120	49	133
	105	149	Bobs	70	136	143	108	153
	105	149	Little Club	66	149	149	52	153
Penny	74	90	Bobs	70	136	113	60	111
Haynes Bluestem	79	215	Marquis	46	195	205	24	214
Average	73	171		69	140	156	55	160
Emmer	62	204	Little Club	66	149	177	12	169
Mindum	77	131		66	149	140	2	189
	77	131	Marquis	46	195	163	14	151
	77	131	Velvet Chaff	105	149	140	17	123
Marquis	46	195	Emmer	62	204	200	18	210
Velvet Chaff	105	149		62	204	177	28	149
Average	74	157		68	175	166	15	165

For total culm length, 6 of the 11 varietal crosses showed an increase over the parental average and 5 showed a decrease. The averages for culm length of the F_1 crosses and of their parents are practically identical when the results of all crosses are considered together. This makes it doubtful whether the increases of the F_1 crosses over the parental averages are the results of the vigor due to crossing or are due to some other experimental factor.

TABLE V.—*Average yield of grain per plant of F_1 wheat crosses and their parents*

Name of one parent.	Number of individuals.	Yield.	Name of other parent.	Number of individuals.	Yield.	Average yield of parents.	F_1 cross.		Per centage of increase with parental average as basis.
							Number of individuals.	Yield.	
		Gm.			*Gm.*	*Gm.*		*Gm.*	
Marquis	15	1.9	Penny	36	2.4	2.2	18	2.7	33
	15	1.9	Bobs	59	3.0	2.5	65	3.3	32
Velvet Chaff	38	1.5	Penny	36	2.4	2.0	28	2.5	25
	38	1.5	Bobs	59	3.0	2.3	92	2.9	26
Penny	36	2.4		59	3.0	2.7	23	2.8	4
Haynes Bluestem.	47	2.4	Marquis	15	1.9	2.2	18	2.5	14
Marquis	15	1.9	Little Club.	46	2.2	2.1	45	2.3	10
Velvet Chaff	38	1.5		46	2.2	1.9	37	2.5	32
Average	30	1.9		45	2.5	2.2	41	2.7	23
Little Club	46	2.2	Emmer	48	1.1	1.7	9	.3	.....
	46	2.2	Mindum	49	2.1	2.2	1	1.0	.....
Marquis	15	1.9		49	2.1	2.0	13	.3	.....
Velvet Chaff	38	1.5		49	2.1	1.8	8	1.1	.....
	38	1.5	Emmer	48	1.1	1.3	23	.5	.....
Marquis	15	1.9		48	1.1	1.5	18	.6	.....
Average	33	1.9		49	1.6	1.8	12	.6	.....

For average yield of grain per plant, six of the eight variety crosses yielded more than either parent, and all variety crosses yielded more than the parental average. Marquis × Bobs and Velvet Chaff × Little Club exceeded the parental average 32 per cent in yield of grain per plant.

With the exception of crosses between common wheat and Little Club the average yield of grain per plant of the species crosses was less than that of the lower-yielding parent. This is due to the fact that the F_1 plants had a high percentage of barren florets.

STERILITY IN SPECIFIC CROSSES

The occurrence of sterility in wheat specific crosses has been reported by several workers. Tschermak (*15*), after several years of hybridization work, found that hybrids of *Triticum dicoccum* and *T. compactum* or vulgare varieties were only partially fertile. Hybrids of *T. durum* with *T. compactum* or *T. vulgare* varieties were classed as fully fertile. Ster-

ility is mentioned by Kezer and Boyack (*11*) as occurring in the F_1 generation of the cross Fultz Mediterranean by Black Winter Emmer. In crosses between Algerian Macaroni and Algerian bread wheats, Freeman (*9*) reports that the F_1 generation developed normally but in the F_2 generation all degrees of sterility appeared from complete sterility to complete fertility.

Hayes, Parker, and Kurtzweil (*10*) crossed varieties of *Triticum vulgare* with varieties of *T. durum* and *T. dicoccum*. The parental varieties showed an average of 4 per cent of barren florets. The F_1 crosses of varieties of durum with varieties of vulgare and the reciprocals showed a barrenness of 47 per cent. The F_1 crosses of *T. dicoccum* crossed with varieties of vulgare showed 26 per cent barrenness and the reciprocal 29 per cent. The results are not in agreement with the conclusions of Tschermak (*15*).

In the present experiment a count was made of the total number of outer florets per plant and the number of these which were barren. From these data the percentage of barren florets was computed. (Table VI.)

TABLE VI.—*Barrenness of outer florets in wheat varieties and F_1 crosses*

Variety or cross.	Number plants considered.	Percentage of barren outer florets.
Marquis	15	18
Velvet Chaff	38	21
Penny	34	15
Haynes Bluestem	47	18
Bobs	59	17
Little Club	47	25
Emmer	49	18
Mindum	44	19
Average	42	19
Marquis×Penny	18	17
Marquis×Bobs	65	14
Velvet Chaff×Penny	27	15
Velvet Chaff×Bobs	93	13
Haynes Bluestem×Marquis	18	17
Penny×Bobs	22	15
Marquis×Little Club	57	17
Velvet Chaff×Little Club	37	14
Average	42	15
Marquis×Emmer	18	73
Velvet Chaff×Emmer	23	67
Little Club×Emmer	10	86
Average	17	75
Marquis×Mindum	13	88
Velvet Chaff×Mindum	8	67
Little Club×Mindum	2	54
Average	8	70

The parental varieties showed an average of 19 per cent barren florets. Intercrosses of vulgare varieties and crosses between Little Club and vulgare varieties showed an average of 15 per cent barren florets. The F_1 crosses of Marquis, Velvet Chaff, and Little Club with Emmer gave an average of 75 per cent barrenness. These same common varieties and Little Club crossed with Mindum showed a barrenness of 70 per cent. These data confirm the results of Hayes, Parker, and Kurtzweil (*10*) and show conclusively that in some cases F_1 crosses between varieties of *T. vulgare* and *T. durum* or *T. dicoccum* are highly self-sterile.

DISCUSSION OF RESULTS

It has been pointed out by East and Jones (7) that the increase in productivity of a cross is due to an increase in the number of growth factors of which the maximum number can be obtained only in a heterozygous condition. In a crop such as corn, this heterozygous condition is kept up by cross-fertilization. Selfing corn varieties reduces the heterozygosity and consequently the vigor. In wheat the continued selfing natural to the crop has brought about a condition of homozygosity.

In the present experiment all varietal crosses gave an increase in seed weight as an immediate effect of cross-pollination. An increase is also shown in the F_1 crosses for average yield of grain per plant as compared with the parental average. The increase ranged from 4 per cent in Penny × Bobs to 32 per cent in Marquis × Bobs. Before attempting to utilize the vigor of the heterozygous condition by growing F_3 and F_4 generation crosses as the commercial crop, it seems logical to combine in one variety the maximum number of growth factors possible. When the possibilities of combination have been exhausted and a variety, or a series of varieties, has been secured which contains this maximum number of growth factors, it may be desirable to follow out the suggestion of Anderson (*1*). This method probably could not be used to advantage except under intensive farming conditions. In case one desired to use such a method it is logical to assume that the more desirable crosses to make are those which show the greatest increase in yield of grain in the F_1 generation.

There is an indication that the increased productivity of the heterozygous condition is a factor which must be considered in comparing F_3 and F_4 lines for yielding ability. In the cross Marquis × Bobs the F_1 generation showed on an average a 32 per cent increased yield of grain per plant as compared with the average of the parents and a 10 per cent increase as compared with the higher-yielding parent. In the F_2 generation of such a cross it is highly probable that some of the most vigorous plants will be those with the greatest degree of heterozygosity. These heterozygous individuals will produce F_3 progeny the vigor of which likewise will be partially due to the heterozygous condition. If the F_3 lines are classified on a basis of their yielding ability, some of these heterozygous lines will

be included as the best yielders. In subsequent generations as the lines become homozygous their productivity may decrease. In generations beyond the F_5 the heterozygous condition of the population rapidly disappears.

A method of breeding which, according to Babcock and Clausen (*3*), has been used by the Svalöf Station, seems worthy of wider application. As self-fertilized crops approach homozygosis rapidly in generations following a cross, it is suggested that a cross be made between varieties selected because of the desirable characters which they possess. After 6 to 10 years have elapsed, during which time progeny of the cross has been grown in bulk plots, selection of individual plants may be made with the assurance that a high percentage of these plants will give homozygous progeny. While this system requires some length of time before results are obtained, it requires a minimum of labor.

When making crosses with the hope of increasing yield through a recombination of the desirable factors of both parents, the parents will naturally be selected on the basis of their yielding ability. The chances of favorable recombinations of yield factors in generations following a cross will presumably be greater when dealing with a cross which shows maximum increased yield over the parents in the F_1 generation. Genetic linkage, however, may make certain combinations difficult or impossible.

The sterility of the specific crosses, with the exception of crosses of varieties of *Triticum vulgare* with Little Club, is partially or wholly responsible for the low grain yield of the crosses as compared with the parental averages. The fact that Little Club behaves in every way as a variety of *T. vulgare* agrees with the view of Tschermak (*15*), who believes that *T. compactum* and T. *vulgare* are closely related. Little Club crosses readily with varieties of vulgare. When Little Club or varieties of vulgare are crossed with Emmer or Mindum, the same high degree of sterility is shown.

SUMMARY OF RESULTS

(1) An increase in seed weight was obtained in all varietal crosses as an immediate effect of cross-pollination. The only significant difference shown by the immediate hybrids of specific crosses was a decrease in seed weight obtained in Velvet Chaff × Mindum.

(2) In the F_1 generation some of the hybrids exceeded the parental average in height of tallest culm, and in total culm length others showed a decrease. In all varietal crosses the F_1 hybrid exceeded the parental average in yield of grain per plant, and six out of eight crosses exceeded the yield of the better parent.

(3) Crosses between Little Club and varieties of *Triticum vulgare* gave results similar to those of crosses between vulgare varieties.

(4) The F_1 generation of Emmer or Mindum crossed with varieties of *Triticum vulgare* or with Little Club showed a high degree of sterility.

The average percentage of barren florets of the parental varieties was 19. The average percentage of barren florets of the F_1 varietal crosses, including crosses of Little Club with vulgare varieties, was 15. The vulgare-Emmer and Little Club-Emmer crosses produced 75 per cent barren florets, while an average of 70 per cent of barren florets was obtained from the durum-vulgare and durum-Little Club crosses.

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TEMPERATURE AND HUMIDITY STUDIES OF SOME FUSARIA ROTS OF THE IRISH POTATO [1]

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INTRODUCTION

The ability of *Fusarium oxysporum* Schlect. to cause a rot of the potato tuber has been clearly demonstrated by a number of workers. The influence of temperature on this disease has been reported in a number of papers, but the experimental evidence as a whole is rather meager, usually only extreme temperatures being used. The effect of moisture on the progress of the disease, except under conditions of extreme dryness or saturation, has received practically no attention. It was with the purpose of determining the relation of temperature and humidity to the progress of potato tuber-rots caused by Fusaria that the following work was undertaken.

HISTORICAL

The association of Fusaria with storage-rots of the Irish potato (*Solanum tuberosum* L.) has been a matter of common observation by most workers in plant pathology from 1842 to date. Several species of the form genus Fusarium Link have been described as causes of potato tuber-rots, by Von Martius (*12*), Reinke and Berthold (*18*), Schacht (*19*), Pethybridge and Bowers (*14*),[2] Longman (*10*), and Sherbakoff (*20*). The fact that Fusarium species could produce a rot of the tuber was demonstrated by Pizzigoni (*15*) and Wehmer (*24, 25*), who described the species they worked with as *Fusarium solani* (Mart). Frank (*6*), De Bary (*2*), and others considered that the Fusaria were unable to produce a rot of the tuber. In most of the earlier papers, *F. solani*, or some species thought to be a synonym of it, was given as the causal organism.

Owing to the absence of clearly defined species in all the literature previous to Appel and Wollenweber's (*1*) monograph on the form genus Fusarium in 1912, no attempt will be made to review in detail the earlier reports of potato tuber-rots caused by Fusaria.

Fusarium oxysporum was considered by Wollenweber (*27*) to be a strictly vascular parasite producing a wilt of the potato vine but not

[1] Published with the approval of the Director of the Nebraska Agricultural Experiment Station. The paper is based upon experimental work undertaken at the Michigan Agricultural College in 1914–15, and at the University of Wisconsin in 1916–17.

[2] Reference is made by number (italic) to "Literature cited," p. 77-79.

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causing a rot of the tuber. Carpenter (*4*) in 1915 was the first to report successful infections by inoculations with pure cultures of *F. oxysporum*. He made these by dipping wounded tubers in a water suspension of spores, wrapping in oiled paper and keeping them at controlled temperatures ranging between 17° and 30° C. No detailed experiments were reported except in this saturated atmosphere. He noted, however, that either a dryrot or a wetrot was produced, according to the temperature and humidity used. He concluded that a constant storage temperature below 50° F. (10° C.) would prevent the action of *F. radicicola* Wollenw., *F. eumartii* Carp., and *F. oxysporum*. Previous to this work of Carpenter's, Smith and Swingle (*22*), in 1910, described a bundle blackening and a dry endrot of the tuber as two stages of the same disease. They attributed this to a Fusarium for which they accepted the name *F. oxysporum* as first applied to it by Schlechtendahl (*21, p. 139*). They noted that the disease continued in stored potatoes and that when potatoes were stored in warm rooms, either moist or dry, they became badly diseased, whereas those stored in cool places kept much better. They did not differentiate this species of Fusarium from others occurring on the potato, and no inoculation experiments were recorded. Manns in 1911 (*11*), working with the same disease, stated that the "dormant internal infection" under improper storage conditions becomes so active as to cause a high percentage of dryrot. He noted that the disease was favored by high temperature and considerable moisture. At 36° to 40° F. (2° to 3° C.) the disease made no progress, at 45° to 55° F. (7° to 12° C.) it developed gradually and caused considerable rot, especially when accompanied by high humidity. He made no mention of pure culture inoculations on tubers or morphological studies.

Jamieson and Wollenweber in 1912 (*8*) described a dryrot of the potato tuber caused by a species of Fusarium which they named *Fusarium trichothecioides* Wollenw. They made inoculation experiments and found the most rapid penetration of the tuber to take place at 10° to 12° C. in an atmosphere of low humidity. Rotting took place at the high humidities but not as rapidly. Wilcox, Link, and Poole (*26*) published on a dryrot of the potato tuber caused by a Fusarium which they called F. *tuberivorum* W. and L. but which was undoubtedly the *F. trichothecioides* previously described by Jamieson and Wollenweber (*8*). They found that a temperature of 8° to 10° C. was only slightly inhibitive to the growth of the fungus and that when potatoes infected with the organism were stored at this temperature, the most rapid decay took place when the humidity was high. Pratt (*17*), working with the same disease, found that temperatures ranging from 12° to 25° C. were favorable for the progress of the disease and that dryrot did not develop at temperatures below 2° C. He concluded from storage experiments that in a dry, well-ventilated storage house losses would be very slight at temperatures from 2° to 4° C.

Link (*9*), making comparative studies of *Fusarium oxysporum* and *F. trichothecioides* found that both were capable of producing a rot of the potato tuber and that *F. trichothecioides* produced a typical dry-rot. *F. oxysporum* produced a softrot of the whole tuber except under cold, dry conditions, when a dryrot was produced. He ran his experiments at controlled temperatures ranging from 1° to 30° C. in an almost saturated atmosphere.

Pratt (*16*) found that *Fusarium radicicola* behaved much the same as *F. oxysporum*, and he concluded from storage experiments that the tuber-rot caused by this organism does not make any progress in storage at a temperature of 48° F. (8.8° C.) or below.

In general, then, it can be said that a high temperature favors the production of tuber-rots by all three of these Fusaria, although *Fusarium trichothecioides* appears to be able to produce a rot at lower temperatures than the other two. High humidities also appear to favor the production of tuber-rot. With the exception of the paper of Jamieson and Wollenweber (*8*) all the evidence points toward an increase in rotting with an increase in humidity.

TEMPERATURE RELATIONS IN PURE CULTURES

A review of the literature shows a general conformity of results regarding the relation of temperature to the growth of *Fusarium oxysporum*. Link (*9*) by making dry-weight determinations of growth in liquid media found 30° C. to be the optimum for growth. Edson and Shapovalov (*5*), working with Petri-dish cultures, obtained the same optimum. They reported a maximum temperature of 37 C., where the spores changed to chlamydospores; they did not observe growth at 5° C. Humphrey (*7*) gives 4° C. as the minimum temperature for certain strains of *F. oxysporum*.

The writer, working with three strains of *Fusarium oxysporum* and using the same methods for measuring growth, obtained somewhat similar results to those reported by Edson and Shapovalov (*5*). The minimum temperature for growth was 9.5° C., no growth taking place at the next lower temperature of 7° C. The maximum temperature was 37.5° C., where there was a very slight growth.

Fusarium trichothecioides is apparently unable to grow at 30° C., which is the optimum temperature for *F. oxysporum*. Link (*9*) found the greatest growth of *F. trichothecioides* in liquid potato extract media at the end of 20 days to take place at 12° C., with no growth present at 30° C., although the organism was capable of living in the potato tuber at that temperature. Edson and Shapovalov (*5*) obtained a much higher optimum for *F. trichothecioides;* they found the greatest growth took place at 25° C., with a sharp drop to the maximum temperature at 30° C., where germination of spores took place but no growth of mycelium.

The writer, working with two strains of *Fusarium trichothecioides* in Petri dishes, found 25° C. to be the optimum temperature, and with one strain he was able to obtain slight growth, 7 mm. in diameter, at the end of one week at 30° C. At 5° C. germination took place and there was slight growth.

The optimum temperature for *Fusarium radicicola* was 30° C., the same as for *F. oxysporum*. The minimum was at 5° C., where a very slight growth was produced in 10 days. At 35° C. the growth was greater than with *F. oxysporum*, although the rate of growth was slower. Edson and Shapovalov (5) report a similar optimum temperature, with germination but no growth at 5° C. They found that at 39° C. a transformation from normal spores to chlamydospores took place.

In general it can be said that at 25° C. the growth for all three species is nearly equal, *Fusarium oxysporum* and *F. radicicola* increasing in growth up to 30° C. and *F. trichothecioides* decreasing. The minimum temperature for *F. oxysporum* is higher than for the other two, and in general *F. trichothecioides* appears to be more tolerant of the lower temperatures than the others.

Preliminary experiments, using liquid media and determining the growth by dry weights, have been conducted with a number of strains of these three species. While on certain media the results have in general corroborated the foregoing cardinal points for growth, they indicated that these cardinal points may vary with the medium used. For instance, with an nutrient solution made up of ammonium nitrate (NH_4NO_3), potassium phosphate (KH_2PO_4), magnesium sulphate ($MgSO_4$), ferric chlorid ($FeCl_3$), and sucrose, the results compared well with those obtained on agar in Petri dishes, With a nutrient solution made up similarly to the potato extract medium used by Link (9), the total growth at the higher temperatures was considerably less than the growth obtained in the first nutrient solution, while at the lower temperatures the growth was much greater. The optimum temperature for growth of *Fusarium trichothecioides* in the first nutrient solution was 25° C., with no growth taking place at 5° C. With Link's potato-extract medium the optimum lay between 15° and 20° C., and there was weighable growth at 5° C. These results would possibly account for the considerable discrepancy between the results obtained by Link (9) with liquid media and those obtained by the writer and by Edson and Shapovalov (5) with agar cultures.

EXPERIMENTAL INFECTION OF TUBERS

The cultures used in the following experiments, with their origin, are listed below. In practically all cases the various strains of the same species behaved alike. Several other strains of *Fusarium oxysporum*, isolated by the writer, were also used in the experiments in addition to the ones listed below.

No. 1.—*Fusarium oxysporum*, isolated by the author from browned vascular bundles of potatoes and identified by H. W. Wollenweber and numbered at Washington as 3377.

No. 8. *Fusarium oxysporum*, obtained from C. W. Carpenter of the United States Department of Agriculture, No. 3395.

No. 32.—*Fusarium oxysporum*, obtained from G. K. K. Link, of the University of Nebraska, as No. 3345a.

No. 28.—*Fusarium trichothecioides* obtained from G. K. K. Link.

No. 31.—*Fusarium trichothecioides*, obtained from A. C. Pratt of the United States Department of Agriculture.

No. 29.—*Fusarium radicicola*, obtained from A. C. Pratt and numbered 716.

METHODS

In all inoculation experiments with tubers, potatoes which were of one variety, of the same age, and had been kept under the same storage conditions were carefully selected for uniformity of size, type, and freedom from wounds. The stem ends always were cut and examined for natural infection, and all tubers showing vascular discoloration were discarded. The tubers were always treated with formaldehyde or mercuric chlorid and washed in sterile distilled water.

The inoculations were made by wounding the epidermis, usually by stabbing to a depth of 3 mm. with a sterile scalpel. The inoculum was introduced in various ways as outlined in the experiments.

EXPERIMENT 1, DECEMBER, 1915.—Potato tubers of the Up-to-Date variety were inoculated by wounding the tubers and then dipping them in a water suspension of spores, wrapping in sterile waxed paper, and placing in moist chambers at 25° C. Controls were treated in the same way, being dipped in sterile water. Results were taken 18 days later.

Set No. 1. Four tubers inoculated with *Fusarium oxysporum* No. 1. All tubers completely rotted. The two control tubers remained sound.

Set No. 2. Four tubers inoculated with *Fusarium oxysporum* isolated from infected tubers in storage. All tubers showed a complete wetrot; the tissue was soft and of a light brown color; a large cavity was present in each tuber containing masses of white mycelium. At the point of inoculation there was a granular mass of hyphae and starch grains separated from the rest of the tissue. Control tubers remained healthy.

Set No. 3. Four tubers inoculated with *Fusarium oxysporum*, isolated from wilted potato vines. All tubers showed a dark brown dryrot progressing only a short distance from the point of inoculation. Controls remained healthy.

Reisolations were made from all the rotted tubers, and *Fusarium oxysporum* was recovered in every case. No bacteria or secondary invaders were found in any of the tubers. These results show that *F. oxysporum* is capable of producing a rot of the tuber in a saturated atmosphere at

25° C. The characteristic rot under these conditions is a soft wetrot with no sharp line of demarkation between the healthy and diseased tissue. The organism appears to be unable to attack whole starch grains, which accumulate in a granular mass with the mycelium, as in set 2. The tuber-rot under these abnormal conditions is not typical of the rots usually found in storage.

EXPERIMENT 2, FEBRUARY 23, 1916.—Further tests were conducted at the same temperature but with a lower relative humidity to test the ability of the organism to cause a rot under conditions not so adverse for the host as in the previous experiment. Tubers of the Up-to-Date variety were inoculated by wounding and then placing a little of the fungus mycelium and spores in the wound. The tubers were then placed in a sterile moist chamber but were not wrapped in paper. Controls were treated and wounded in the same way. The experiment was run at 25° C. Twelve different strains of *Fusarium oxysporum* were used for the inoculations, two tubers being used for each strain. Results were taken after five weeks.

In only one case had the rot extended three-fourths of the length of the tuber. In all the other tubers there was only a slight rotting extending for a short distance from the point of inoculation. The controls remained sound in every case. The tubers were in a saturated atmosphere at the beginning of the experiment, gradually becoming drier until at the end the tubers were considerably dried out. Compared to the preceding test the amount of rotting was very slight, and its inhibition may be directly attributed to the dryness of the air. The slight amount of rot around the point of inoculation would indicate that the fungus progressed a short distance into the tuber at the beginning of the experiment when the humidity was high but was unable to advance further under the drier conditions. This would indicate that the rotting of tubers already started could be checked by submitting the tubers to lower humidities.

EXPERIMENT 3, APRIL 26, 1916.—A further test on the relation of humidity of the atmosphere to the rot of the tuber was started. The inoculations were made as in the previous experiment, and the same variety of potatoes was used.

Set No. 1. The inoculated tubers were placed in sterile chambers, and moist filter paper was placed in the chambers at the start of the experiment to produce a favorable humidity for the initial penetration of the tuber.

Set No. 2. The tubers were placed in moist chambers in which the atmosphere was kept saturated throughout the experiment.

Both sets were kept at a temperature of 25° C. The results were taken after seven weeks. (Table I.)

TABLE I.—*Comparative amount of rot produced by Fusarium spp. under different conditions of relative humidity*

Strain.	Set No. 1.	Set No. 2.
F. oxysporum No. 1	One-third rotted	Entirely rotted.
F. oxysporum No. 8	2-mm. rot	Do.
F. hyperoxysporum	One-third rotted	Half rotted.
Control	Healthy	Healthy.

In all cases where rotting was present the starch grains were not corroded. Culture No. 8 seemed to have a much slower initial growth than the others, thus showing a greater difference between the two sets. In general, it can be clearly seen that the rotting was much greater in set No. 2, where the atmosphere was saturated throughout the experiment. Although the organisms were capable of starting a rot under the moist conditions at the start of the experiment in set. No. 1, they were later considerably checked under the drier conditions.

EXPERIMENT 4, MARCH 15, 1917.—Further infection experiments were started under conditions in which the relative humidity of the atmosphere was controlled by the use of various concentrations of sulphuric acid. Previous experiments conducted at the Michigan Agricultural Experiment Station in 1915 and described under experiment 5, in which the relative humidities were carefully controlled, produced very good results with *Fusarium oxysporum*.

The apparatus used in experiment 5 was not available in 1917, so the relative humidities used in experiments 4 and 4A were determined from the tables given by Stevens (*23*). One-quart Mason jars were used, in which were hung small wire baskets containing the tubers, the acid being placed in the bottom of the jar. Tubers of the Rural New Yorker variety were inoculated as in the preceding experiments. They were then placed in the baskets in the sterilized jars and were sealed with paraffin and placed at the desired temperatures. The experiment was run in duplicate. Three strains of *Fusarium oxysporum*, two of *F. trichothecioides*, and one of *F. radicicola* were used for the inoculations. The temperatures used were 5°, 9°, 16°, and 25° C. While these temperatures varied somewhat during the experiment, the extremes did not in any case overlap. The relative humidities obtained by using sulphuric acid remained fairly constant throughout the experiment. One hundred cc. of each of the acid solutions were used for each jar. At the close of the experiment the specific gravity of the solutions was taken, and the calculated humidity at this time was compared with that at the start, with the result that the one having 1.5 per cent relative humidity had changed to 3.6 per cent, the 33 per cent to 49 per cent, and the 66.5 per cent to 74 per cent. These variations were not considered great enough to cause conflicting results.

The results were taken after seven weeks, and the penetration of the tubers was measured in millimeters, as shown in Table II. The number of individuals was so small that slight discrepancies in the tabulated results are found. *Fusarium trichothecioides* produced a slight rot at lower temperatures than *F. oxysporum* but did not produce as extensive a rot at the higher temperatures. At 5° C. the only rotting found was with one strain of *F. trichothecioides*, at 100 per cent humidity. No rotting was found at the temperature of 9° at the lower humidities, but there was slight rotting at this temperature at the higher humidities, especially with *F. trichothecioides*. It is noticeable that at 9° with the relative humidities of 66 and 100 per cent, the amount of rotting is greater than at the increased temperature of 16°, with the relative humidities of 1 and 33 per cent. The same comparative results are found between the amount of rotting taking place under the several humidities at a temperature of 16° and of 25°. The results do not conform with the report of Jamieson and Wollenweber (*8*), that penetration of the tuber by *F. trichothecioides* is favored by low humidities. The work by Link (*9*) and Wilcox, Link, and Poole (*26*), however, would indicate that more rapid rotting takes place in an atmosphere of high humidity, thus agreeing with the results shown in this experiment. The results of *F. oxysporum* accord well with those obtained in experiment 3.

TABLE II.—*Extent of penetration of tubers in experiment 4*

Temperature.	Approximate relative humidity.	*Fusarium oxysporum.*			*Fusarium trichothecioides.*		*Fusarium radicicola,* Strain 29.
		Strain 1.	Strain 8.	Strain 32.	Strain 28.	Strain 31.	
°C.	*Per cent.*						
5.....	1	0	0	0	0	0	0
	33	0	0	0	0	0	0
	66	0	0	0	0	0	0
	100	0	0	0	0	3 mm.	0
9.....	1	0	0	0	0	0	0
	33	0	0	0	0	0	0
	66	0	1 mm.	0	0	1 mm.	0
	100	0	1 mm.	0	1 mm.	3 mm.	1 mm.
16.....	1	0	0	0	0	0	0
	33	1 mm.	0	0	0	0	0
	66	2 mm.	3 mm.	0	1 mm.	1 mm.	0
	100	10 mm.	10 mm.	0	1 mm.	1 mm.	2 mm.
25.....	1	5 mm.	10 mm.	1 mm.	0	0	1 mm.
	33	10 mm.	15 mm.	3 mm.	0	2 mm.	6 mm.
	66	20 mm.	½ rot.	½ rot.	1 mm.	25 mm.	6 mm.
	100	½ rot.	Complete rot.	¾ rot.	1 mm.	½ rot.	Complete rot.

EXPERIMENT 4 A, MAY 16, 1917.—In order to check up the possible error due to differences in the age of the tubers used in the various tests, the following experiment was started. New tubers of the Bliss Triumph variety were used in comparison with tubers of the same variety that had

been kept in cold storage from the previous year. The experiment was conducted in the same way as experiment 4, and the same cultures of *Fusarium oxysporum*, *F. trichothecioides*, and *F. radicicola* were used for inoculations. Only two temperatures were used, 13.5° and 25° C., as well as two humidities, 33 and 100 per cent at each temperature. The results shown in Table III were taken after six weeks.

As in experiment 4 the rotting was much greater at the high temperatures and the high humidities. At the lower temperature of 13.5° C. there was no distinct difference between the amount of rotting in the old and new tubers, due to the very slight penetration at this temperature. In the old tubers at 25° the infection in every case had been rapid and the rotting had progressed much further than in the new tubers. These results support the statement of Bisby (3) that old tubers are more susceptible to rot than new tubers.

TABLE III.—*Extent of penetration of old and new tubers*

Temperature.	Approximate relative humidity.	Tubers.	*Fusarium oxysporum.*			*Fusarium trichothecioides.*		*Fusarium radicicola*, Strain 29.
			Strain 1.	Strain 8.	Strain 32.	Strain 28.	Strain 31.	
°C.	*Per cent.*							
13.5	33	New.	2 mm.	5 mm.	1 mm.	4 mm.	2 mm.	5 mm.
		Old.	2 mm.	2 mm.	1 mm.	7 mm.	10 mm.	5 mm.
	100	New.	5 mm.	5 mm.	1 mm.	2 mm.	2 mm.	5 mm.
		Old.	5 mm.	4 mm.	1 mm.	5 mm.	6 mm.	5 mm.
25	33	New.	20 mm.	2 mm.	3 mm.	4 mm.	5 mm.	¼ rot.
		Old.	Contaminated.	15 mm.	15 mm.	¼ rot.	5 mm.	¼ rot.
	100	New.	Contaminated.	¼ rot.	Contaminated.	2 mm.	2 mm.	¼ rot.
		Old.	5 mm.	½ rot.	15 mm.	10 mm.	½ rot.	Complete rot.

EXPERIMENT 5, 1915.—The results of earlier experiments having indicated that the influence of the relative humidity was nearly as great as that of temperature, it was decided to run a more complete test on the effect of the relative humidities at different temperatures. Since no apparatus was available by which the relative humidity and temperature could be controlled at will, it was necessary to construct one.

The principle employed in experiment 4 of using sulphuric-acid solutions of varying specific gravity in a closed chamber to obtain the different relative humidities was not used in this test. In preliminary experiments conducted in the same way as experiments 4 and 4A, the infection usually resulted in a softrot which gave good comparative results, but the type of rotting was not similar to that usually found in storage. Cultures from these softrots invariably yielded the *Fusarium* sp. used in the inoculation, and no bacteria were present in any case. Apparently

the absence of any aeration was the cause of this abnormal type of rotting, and the following method was devised to allow for aeration.

The principle finally decided upon was that of passing a current of air, kept at a constant pressure, through sulphuric-acid towers and then over calcium chlorid and sodium hydrate. This gave a constant stream of dry, sterile air. The air was then passed over sterile water to bring it to a desired humidity. The amount of water necessary for a given humidity was determined by trials, and the air was then passed into the jars containing the tubers. An outlet was provided at the bottom of the jar. These jars were connected separately with the current of air and not in series. Relative humidities were obtained and used throughout the experiment as follows: 1, 30, 70, and 100 per cent. These relative humidities were used at three different temperatures—9°, 12.5°, and 25° C. The set at 25° was placed in an incubator in the laboratory, the set at 12.5° was placed in a special low temperature incubator, and the set at 9° was placed in a well-insulated ice box. Each of these temperatures was maintained within a variation of 2° throughout the experiment. In this way four gradations of humidity at each of three temperatures were obtained. The method provided the tubers with sufficient aeration and secured sterile conditions throughout the experiment, since the jars containing the tubers were not moved or opened until the end of the period.

The humidity readings were taken by the wet- and dry-bulb method, the thermometers being inserted into the stream of air at the entrance to the jar. The readings were found to vary, and at least 10 trial readings were taken for each jar after the preliminary determinations were made and the apparatus was set up. These readings ranged as follows: 1 to 10 per cent, 20 to 40 per cent, 60 to 80 per cent, and 90 to 100 per cent. These were the greatest extremes found; and since a knowledge of the approximate relative humidity is all that is necessary in an experiment of this kind, these readings were taken to be sufficient, inasmuch as they showed a gradual gradation from approximate dryness to saturation. The ranges given above simply denote the possible error due to the method of taking the readings. The humidity necessarily remained constant, since the temperature, water surface, and air pressure were constant. It was found to be impossible to use the wet- and dry-bulb method to determine the relative humidity at the lower temperatures. The changes of temperature caused by opening the door to make the determinations were found to change the readings. Therefore the sets at 9° and 12.5° C. were installed temporarily at 25°, the preliminary determinations were made, and the readings were taken at that temperature and corrections made by the use of psychometric tables (*13*).

The large battery jars were fitted with wire screen supports, and six tubers were used in each jar—four inoculated and two controls. The

control tubers were separated from the inoculated ones by a thin layer of cotton. The entire apparatus was disinfected with formaldehyde gas before the experiment was set up.

The tubers used were of the Up-to-Date variety. They had been kept over winter in a cool cellar, and a few sprouts which had started were removed. They were inoculated by wounding the epidermis and placing several drops of spore suspension in the wound. They were then placed in the jars which were closed with cork tops and paraffined. The inoculations were made with *Fusarium oxysporum* No. 8. The jars were opened up and the tubers examined after five weeks.

Set 1 (9° C.). At 10 and 30 per cent humidity the tubers were all healthy.

At 70 per cent the tubers were sound with no penetration, although there was a slight growth of mycelium on the surface of the tuber at the point of inoculation.

At 100 per cent the condition of tubers was the same as at 70 per cent, except that the external growth of mycelium was greater. All the control tubers of this set remained healthy, and both the controls and inoculated tubers had sprouted.

Set 2 (12.5° C.). At 10 per cent humidity the tubers were healthy. There was no invasion of the tissues.

At 30 per cent, same as above with a slight external growth of mycelium at the point of inoculation.

At 70 per cent the tubers were about the same as at 30 per cent. (Pl. 10, A.)

At 100 per cent invasion of the tissue had taken place for about 2 mm. beyond the wound, causing a slight browning of the tissue. On the surface there was a slight brown discoloration for several millimeters surrounding the point of inoculation and a slight growth of aerial mycelium. All the control tubers in set 2 remained healthy, and both control and inoculated tubers were sprouting normally. (Pl. 10, B.)

Set 3 (25° C.). The control tubers remained healthy and sprouted at 10 and 30 per cent relative humidity, while at 70 and 100 per cent there was a slight disorganization of the tissue around the eyes and the sprouts were all dead. No actual rotting was present or any fungus growth.

With infected tubers at 10 per cent humidity all inoculations were successful and uniform. The fungus invaded the tissue for 2 cm. around the wound. Immediately below the surface at the point of inoculation there was in every case a cavity lined with a white mycelial growth. The tissue surrounding the cavity was of a granular appearance. Examined under the microscope it appeared to be made up of a tangled mass of mycelium and starch grains. A softrot extended out from this area, the tissue being light brown in color and completely invaded by mycelium (Pl. 10, C).

At 30 per cent humidity the rotting took place in the same manner as at 10 per cent, except that the cavity was larger and the rot extended through about 50 per cent of the tuber (Pl. 11, B).

At 100 per cent humidity there was a total rot of all tubers, most of the surface being covered with a white mycelial growth (Pl. 11, C).

The results of this experiment show very clearly that relative humidity plays a very important part in determining the amount of rot produced by *Fusarium oxysporum*. The only rot appearing at the low temperature of 12.5° C. was in an atmosphere of 100 per cent humidity. Even at the high temperature of 25° complete rotting did not take place at the lower humidities. A gradual increase in the amount of rot corresponding to the increase in humidity was present in every case. The fungus can live and sporulate at the lower temperatures and lower humidities used in this experiment but apparently is not capable of penetrating the tubers under these conditions. It can be safely concluded that *F. oxysporum* under good storage conditions is not capable of producing a tuber-rot of great importance.

CONCLUSIONS

(1) *Fusarium oxysporum*, *F. trichochecioides*, and *F. radicicola* are all capable of producing a rot of the potato tuber.

(2) In pure culture the amount of growth of all three species is nearly equal at 25° C., *Fusarium oxysporum* and *F. radicicola* increasing in growth up to 30°, where they produce their maximum growth. The growth of *F. trichochecioides* decreases above 25°, until at 30° very little or no growth takes place. It is more tolerant of the lower temperatures than the other two species.

(3) Preliminary tests with different liquid media would indicate that the cardinal points for growth of these Fusaria vary to some extent with the medium used.

(4) Experimental infection of tubers was produced with all three organisms under various conditions of temperature and relative humidity.

(5) Preliminary tests with *Fusarium oxysporum* indicated that the relative humidity plays a very important part in determining the amount of rotting.

(6) In comparative tests with new and old tubers there is a distinct difference in the amount of rotting under the same conditions. The rotting was much more rapid and progressed much further in the old than in the new tubers.

(7) Comparative tests with all three species at controlled relative humidities from 1 to 100 per cent and at controlled temperatures from 5° to 25° C. proved conclusively that—

(*a*) A temperature of 25° C. is favorable for the production of a tuber rot by *Fusarium oxysporum*, *F. radicicola*, and *F. trichothecioides*.

(*b*) *Fusarium oxysporum* grows more rapidly and produces a more extensive rotting of the tuber than the other two at a temperature of 16° C. and above.

(*c*) *Fusarium trichothecioides* is capable of producing a rot at much lower temperatures than the others, in some cases causing rotting at 5° C.

(*d*) The relative humidity plays a very important rôle in determining the progress of tuber rots and has the same influence on all three species. In every experiment it was noticeable that there was a gradual increase in the amount of rot corresponding to an increase in relative humidity. With a high humidity at a given temperature the rotting was always greater than at a temperature 5° to 10° C. higher but with a low humidity. The Fusaria used can all live and sporulate at the low temperature of 9°, and with low relative humidities, but they are not capable of producing a rot under these conditions.

Inasmuch as the three species of Fusaria used in these experiments represent the common types causing storage-rots of potatoes, it is clear that considerable attention should be given to moisture as well as temperatures where incipient rot occurs in stored tubers. It is also entirely probable that a rotting of the tubers initiated at high temperatures and high relative humidities could be completely checked by submitting the tubers to lower temperatures and lower humidities.

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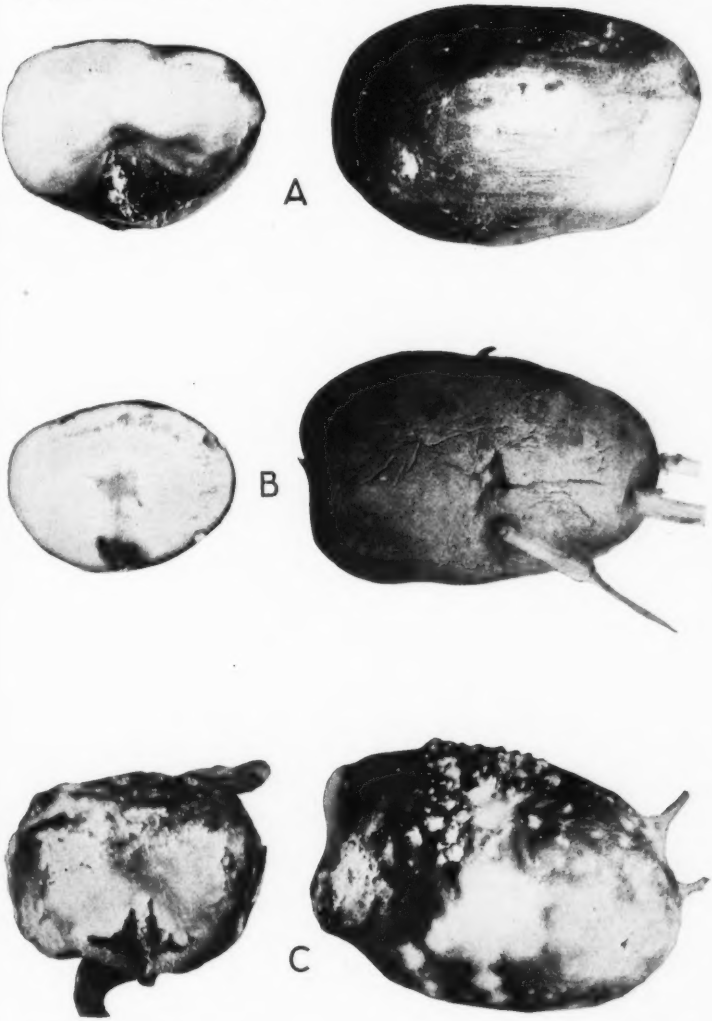
PLATE 10

Tubers inoculated with *Fusarium oxysporum* and kept for five weeks at the following temperatures and humidities:

A.—12.5° C., 70 per cent relative humidity.
B.—12.5° C., 100 per cent relative humidity.
C.—25° C., 10 per cent relative humidity.


A
B
C

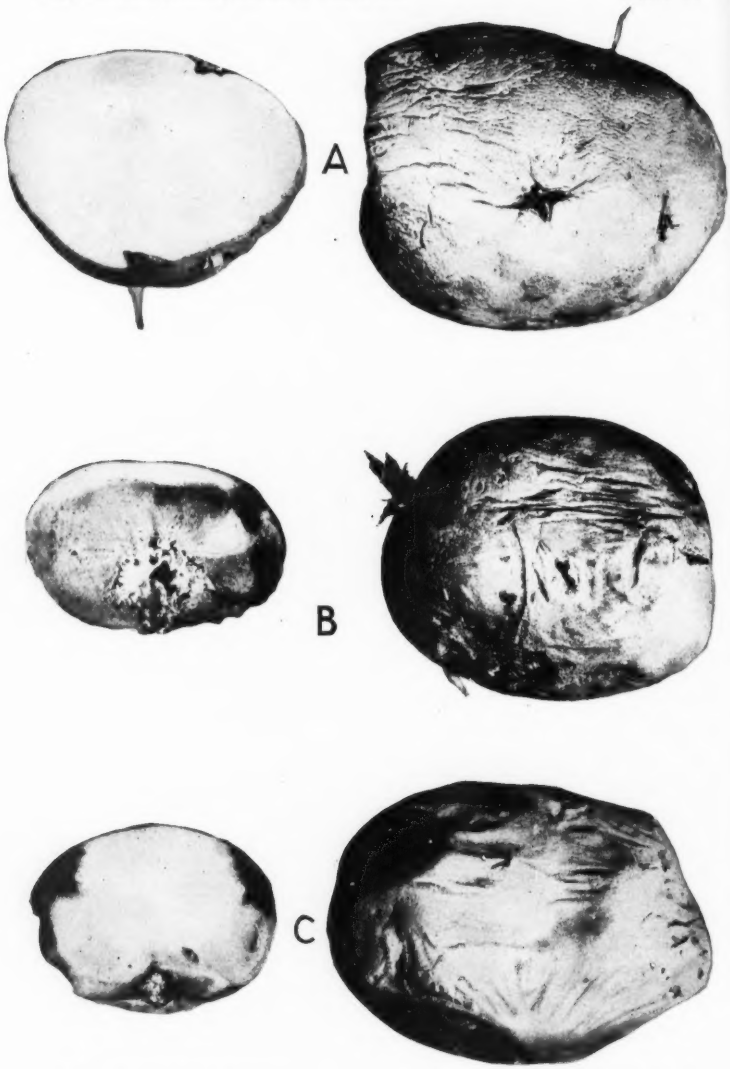

A
B
C

PLATE 11

Tubers inoculated with *Fusarium oxysporum* and kept for five weeks at the following temperatures and humidities:

A.—25° C., 30 per cent relative humidity.
B.—25° C., 70 per cent relative humidity.
C.—25° C., 100 per cent relative humidity.

BLACKLEG POTATO TUBER-ROT UNDER IRRIGATION

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OCCURRENCE AND GENERAL APPEARANCE

A bacterial field decay of the potato tuber, the real nature of which has not heretofore been adequately explained, prevails in certain irrigated sections of the West. In early harvest, when the diseased tubers are apparently free from fungous invasion, the trouble has sometimes been assumed to be "sunscald"; during the winter months it has frequently been taken for a form of freezing injury. In other instances it has been confused with the so-called "jelly-end rot" and attributed either to *Fusarium radicicola* Wollenw. or to *F. oxysporum* Schlecht. It is probable, also, that on superficial examination some such material has been classed as "leak" (*Pythium debaryanum* Hesse), when conditions favored an extremely rapid progress of the decay, whether in the field or in transit.

Specimens of this decay were received by the writers in 1917 and 1918 from Idaho, Nevada, and California. In material received in August the decay was soft and mushy (Pl. 12, A–C). The affected tissues were in part brown to black, but mostly only slightly colored or colorless, though with a darker margin on the border line between the healthy and diseased portions. Disintegration, originating at one end of the tuber, was advancing irregularly over the surface. In some areas the decay was confined to the outer layer, just beneath the epidermis, while in others the deeper tissues also were involved. As a rule, the disease started at the stem end, but occasionally the eye end became infected first (Pl. 12, B). Decaying material usually possessed a disagreeable odor. It is this soft type of the rot which some were inclined to regard as sunscald injury.

Specimens received later in the season, during the months of November and December, presented an entirely different appearance. The affected portions were not mushy, but more or less tough or dry and shrunken (Pl. 12, D). The diseased area was dark brown in color, except when a fresh decay developed under favorable conditions deeper in the tissues. In the latter case it was practically of the same color as the normal flesh of the tuber, but soft and mushy in consistency. When such tubers were cut open and the cut surfaces exposed to the air, the diseased portions turned brown or even black. If the progress of the decay is completely arrested, the trouble may readily be mistaken for

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an inactive stage of jelly-end rot or for an after effect of freezing injury, particularly if the disease has made but little headway. The true nature of such obscure cases of the disease may be revealed with certainty only by a series of cultural studies, coupled with experimental work and field observations.

CAUSAL ORGANISM

Isolations were made from every tuber of each of the four samples received from the West in 1917–18. The results were surprising. In no case was *Fusarium radicicola* obtained; only one tuber yielded *F. trichothecioides* Wollenw. (from Nevada), two yielded Rhizoctonia (from Nevada), two *F. oxysporum* (from California), and a few gave miscellaneous, apparently saprophytic, fungi. Bacteria, on the other hand, were constantly present in the cultures, even when slightly acidulated potato agar was used. Carpenter (*2*)[1] noted the presence of bacteria in jelly-end rot material, but he regarded these organisms as saprophytic, as they probably were. In the writers' cultures, however, the constant prevalence of one type of bacterial colony in the dilution plates was significant and warranted a detailed study of this organism. In the subsequent inoculation experiments with pure cultures it proved to be strongly pathogenic and produced a progressive decay of the tubers as well as a disease of the stems. A study of the cultural and biochemical features of the organism showed them to be fully in accord with the published description of the blackleg bacillus (*5*).

MORPHOLOGY

Short rod with rounded ends, also short chains; 0.5 to 0.9 × 1.0 to 2.2 μ, average 0.6 × 1.8 μ; flagella few, peritrichiate; no endospores and no capsules; stains well in aqueous gentian violet, aqueous methylene blue, aqueous fuchsin, anilin water gentian violet, alkaline methylene blue, and carbol fuchsin.

CULTURAL FEATURES

AGAR STROKE.—Growth moderate, filiform, flat to slightly raised, glistening, smooth, slightly opalescent; white, no odor; consistency slimy to butyrous; one strain distinctly viscid at first, but after a few replatings it lost its viscidity.

POTATO.—Growth moderate to abundant; filiform at first then spreading, slightly convex changing to flat, glistening, smooth to slightly rugose, yellowish white or dirty white; a decided odor of decayed potatoes on the third to fourth day at 22° to 25° C.; consistency somewhat slimy; medium slightly grayed at first, changing later to either plainly gray, or purplish, or brown, or a combination of these shades.

[1] Reference is made by number (italic) to "Literature cited," p. 91–92.

AGAR STAB.—Growth somewhat best at top, abundant, spreading, filiform to slightly papillate.

GELATIN STAB.—Growth best at top, filiform along the line of puncture, liquefaction, beginning on the first day at 20° C., varying in shape from crateriform or funnel-shaped to saccate and broadly infundibuliform, complete in 7 to 12 days.

NUTRIENT BROTH.—Usually slight ring and slight granular pellicle in young cultures, clouding moderate to strong, persistent; medium not discolored, odor absent; sediment compact, granular, somewhat dirty white; one strain decidedly viscid at first, but losing this character after a few replatings.

MILK.—Coagulation and extrusion of whey at 25° C., beginning on the fourth day; coagulum not digested; one strain extremely viscid at first, but losing its viscidity in later replatings; medium not discolored.

ACID PRODUCTION IN MILK.—A slight increase of acidity in milk cultures was noticeable after 24 hours. Two series of tests were made at certain intervals within the period of 20 days, two to three cultures being used on each day for every strain. The average progress of the acidity of three western strains was as follows:

AGE OF CULTURE.	REACTION IN FULLER'S SCALE.
1 day	+12.28
2 days	+13.49
3 days	+22.13
5 days	+28.00
10 days	+32.90
20 days	+41.12

The average reaction of the control tubes was +11.8 Fuller's scale. Two strains received from Dr. W. J. Morse, of the Maine Agricultural Experiment Station, were tested along with the western strains and gave similar reactions, one ("B. sol.") showing 37.0 and the other ("IIIA") 40.75 acidity on the twentieth day. The cultures were grown at 22° to 25° C.

LITMUS MILK.—At 22° to 25° C. bleaching was complete at the end of three weeks; thorough reddening was accomplished in seven weeks.

GELATIN COLONIES.—Growth rapid, form round, edge entire, liquefaction saucer-shaped.

AGAR COLONIES.—Surface colonies; growth rapid, usually round, but occasionally somewhat irregular, flat to slightly raised, entire to slightly undulate, finely granular with an internal ring surrounded by radiate striations; color pearly white, bluish opalescent by transmitted light; maximum diameter of colonies after 2 days 2 mm., after 3 days 4 mm., after 7 days 7 mm., after 14 days 9.5 mm. Buried colonies lens-shaped to nearly spherical, edge entire, color slightly yellow under hand lens.

FERMI'S SOLUTION.—Moderate clouding in 2-day cultures; later growth becomes copious.

COHN'S SOLUTION.—No growth.

USCHINSKY'S SOLUTION.—Growth was somewhat irregular in the ordinary Uschinsky's solution but was uniform and copious in the modified Uschinsky's solution, clouding being very strong on the fifth day.

SODIUM CHLORID IN BOUILLON.—Growth slightly inhibited by 3 per cent and more so by 4 per cent; no growth appeared in 5 per cent tubes until the third day, and only occasional tubes containing 6 per cent were clouded after 5 days. Morse reports no clouding for *Bacillus atrosepticus* Van Hall in concentrations higher than 5 per cent. At the end of two months, when conditions remained unchanged, transfers were made from 6 per cent and 7 per cent sodium-chlorid cultures of the western strains to sterile broth. In 48 hours all the transfers from 6 per cent solutions showed growth, and in three days clouding appeared in the majority of the transfers from the 7 per cent solutions, the remainder being dead.

GROWTH IN BOUILLON OVER CHLOROFORM.—Growth somewhat restrained at first, but increasing gradually. On the fourth day there was a strong and uniform clouding in all cultures.

BEST MEDIUM FOR LONG-CONTINUED GROWTH.—Morse considers that in the case of *Bacillus atrosepticus* neutral beef bouillon is best for this purpose. In the western strains the writers observed that the organisms can live even longer on the agar than on the broth when grown at ordinary laboratory temperature of 22° to 25° C. Their death on agar appears to be primarily associated with drying of the medium, while in broth it seems to be due to certain chemical changes in the substratum and takes place sometime before the liquid dries up completely. Six series of parallel broth and agar cultures were made and tested at different intervals, from 8 to 36 weeks, by making transfers to tubes of sterile broth. It was found that occasional broth cultures showed a somewhat weakened vitality, as demonstrated by retarded clouding, at the age of 16 weeks; some died after the expiration of 20 weeks, and none lived beyond 26 weeks. On the other hand, in no case was the agar culture dead before 26 weeks, and some remained alive even after 36 weeks. The experiment was carried on with 10 cc. of medium in each test tube.

PHYSICAL AND BIOCHEMICAL FEATURES

FERMENTATION TUBES.—Gas and acid production as well as growth in the closed arm was observed with dextrose, lactose, and saccharose. No acid and no gas with glycerin in cultures 1, 3, and 5 days old.

AMMONIA PRODUCTION.—Feeble (tested by Folin's aspiration method).

NITRATES IN NITRATE BROTH reduced to nitrites.

INDOL PRODUCTION.—Positive, but very feeble both in young and old cultures.

TOLERATION OF HYDROCHLORIC ACID AND SODIUM HYDRATE.—The writers' western organism grew in tubes having an initial reaction before

final sterilization of +20 and −20, Fuller's scale, but not in those adjusted to +30 or to −30. Uninoculated tubes held as controls and titrated at the close of a 24-day incubation period showed marked changes from the original reaction, due doubtless in considerable measure to the absorption of gases with resulting chemical change. Tubes calculated for an initial reaction of +30 showed a final reaction of from +20 to +25; those originally +20 were about +15; those −30 were about −10; and those −20 were about −6. Transfers from inoculated tubes calculated for an initial reaction of +30 and above and −30 and below made 24 days after inoculation developed growth in some cases in tubes from +30, but not in those from more acid reactions nor from the alkaline broths.

VITALITY ON CULTURE MEDIA.—Long on bouillon, but still longer on agar.

TEMPERATURE RELATIONS.—In freshly inoculated broth cultures exposed 10 minutes, occasional retardation of clouding began at 45° C.; occasional growth was noted at various points between 46° and 50°; and in no case was growth present after heating above 50°. Optimum temperature for growth about 25°. Maximum temperature for growth between 33° and 35°. Minimum temperature for growth below 5°.

EFFECT OF SUNLIGHT.—Thinly sown agar plates exposed on ice for 30 minutes the latter part of March in Washington, D. C., resulted in 100 per cent killed.

CYTASE PRODUCTION.—Five-day-old 30-cc. broth cultures in 300-cc. Erlenmeyer flasks were precipitated by 160 cc. of 80 per cent alcohol, filtered, and the precipitate dried promptly in the air. The papers containing the dried precipitate were washed with 30 cc. of water, and the washings were received in a flask to which a few drops of toluene and three raw Irish potato disks 15 by 2 mm. were added. The disks gradually assumed a soft, cheesy consistency but did not entirely disintegrate. Microscopic examination showed the cells had lost coherence through softening of the middle lamella. The cellulose lamella and the starch content of the cells showed no evidence of change. Controls with uninoculated broth did not soften the disks.

GROUP NUMBER 221.1113033

The last three points in this group number differ from those given by Morse (*5*) but coincide with the respective figures in Jennison's (*3*) revision, as reported by him at the fourth annual meeting of the Pacific Division of the American Phytopathological Society. The writers feel, however, that this may be largely a matter of interpretation of certain results and not necessarily an indication of actual difference in the organisms. Jennison studied 12 different strains of the blackleg bacillus, including several of Morse's strains; but the results he obtained, apparently,

were identical for all strains. The writers regard their western strains as nonchromogenic, although a certain yellow discoloration in cooked potato cultures might be taken as a suggestion of yellow pigment. They obtained no evidence of diastatic action on potato starch, nor of acid production with glycerin.

It may, therefore, be concluded on the basis of the characters described that the pathogenic bacillus isolated by the writers from a peculiar soft decay of western potato tubers is essentially identical with the organism causing the blackleg disease of potatoes for which Appel's binomial *Bacillus phytophthorus* is regarded to be correct by Smith (7). Morse (5), who was unable to obtain an authentic culture of Appel's strain for his comparative studies of various blackleg organisms, believed that *B. atrosepticus* should be chosen in preference to other names he had under consideration, but stated that—

> There is nothing in the data here presented which bears on the relation between the organism originally described by Dr. Appel (1) as *B. phytophthorus* and the other strains of blackleg bacteria.

EXPERIMENTAL WORK

The pathogenicity of the bacterial organism described above was established by means of the following laboratory, greenhouse, and field experiments.

PLANTING OF THE ORIGINAL MATERIAL

Preliminary to the inoculation work some of the diseased western material was planted in the greenhouse as soon as the isolations were completed. Four tubers were selected and cut in halves so as to make eight seed pieces. Each piece was planted in a separate large pot filled with sterilized soil. Of these seed pieces one decayed completely in the soil before germination, six produced diseased plants, and one produced a plant considerably weakened though not clearly diseased. Some of the affected plants decayed while very young, others grew up to practically normal size, developing blackening of the stem above ground and brown to black lesions on the underground portions. The lower leaves turned yellow, but the upper leaves wilted while green. In two cases the blackening of the stems was very intense (Pl. 13, A) while in the remainder the appearance was less typical of the familiar field symptoms of the disease as it occurs in the eastern sections of the country. Blackening of the pith of the stem developed to the very top in one case. When an affected plant was removed from the pot and the soil was carefully washed off, it could be seen that the infection had spread from the seed piece to the stem (Pl. 13, B). No tubers were produced in this experiment. Healthy sprouting Irish Cobbler tubers were replanted in these pots, but no infection was contracted by this new set of plants.

INOCULATION OF HEALTHY TUBERS IN THE LABORATORY

Over 60 tubers both new and old of the Netted Gem as well as of the Irish Cobbler varieties were inoculated in small lots at different times with three strains of the western decay bacillus. When inoculations were made in wounds of any kind, whether on the side of the tuber or at either end of it, the results were invariably positive. The progress of the decay was much slower when uninjured potatoes were inoculated. In these latter cases the organism penetrated either through the eyes or through the young growing sprouts. If the infected potatoes are removed from the moist chamber after the decay has made considerable headway and are exposed to the dry air of the laboratory, the diseased tissues become shriveled and folded, resembling very closely the original specimens of natural infection (Pl. 14, A, C). Ordinarily if the infected material is kept in moist chambers the decay is soft, mushy, spreading either equally throughout the tissue or sometimes more on the surface of the tubers, and is not confined to their piths as is usual in typical cases of blackleg. The color of the decaying areas ranges from that of the normal flesh to light or dark brown, often with blackish streaks or stripes in younger portions nearer to healthy tissues, but never black throughout. The margin is usually well defined, and there is no gradual transition from dead to sound tissues. This internal appearance changes considerably when tubers are taken from the moist chamber and are exposed to drying. The decay of the bark is then more or less arrested, and the disintegration centers mainly in the pith, so that a more or less sound shell surrounds the centers of the active decay. The diseased tissue is brown to black, the older regions becoming slimy (Pl. 14, B, D). In all cases the decay gives off a very strong putrefactive odor.

PLANTING OF ARTIFICIALLY INOCULATED TUBERS IN THE GREENHOUSE

Seven Irish Cobbler tubers inoculated with the western bacterial organisms and partly decayed were planted in sterilized soil in pots. Four showed subsequently a stem decay and three remained apparently unaffected. One plant became girdled and died early. The disease appeared first on the remaining three plants in the form of black streaks in various positions on the stems, particularly at the leaf petioles. Later on in some instances the entire stalk became black at the base. Tuber-rot did not appear except on one tuber in one of the diseased pots. In this case it was a soft, watery decay, light in color, not typical for blackleg. The causal organism, identical with the original strains, was, however, recovered from this area. Healthy sprouting Irish Cobbler tubers were immediately planted in the same pots in which these specimens were grown. The new plants were very vigorous, and none of them contracted the disease.

INOCULATION OF HEALTHY STEMS IN THE GREENHOUSE

The stems of four young healthy potato plants were inoculated with 24-hour-old broth cultures of the three western strains of the bacillus injected by means of a hypodermic needle. A severe decay with an accompanying blackening resulted in all cases.

FIELD EXPERIMENTS

These experiments were conducted for two successive years at Arlington Farm, Va. In 1919 Netted Gems and Irish Cobblers were used. The tubers were inoculated with the western strains of the blackleg organism a few days before planting. Six whole tubers and 20 halves of the first variety and 9 whole and 19 halves of the second variety were planted. In addition a number of uninoculated pieces of each variety were planted for controls. Planting was done on May 5. One half-tuber seed piece of each variety decayed in the ground. On July 1 one hill from the cut seed of Irish Cobblers was noted to show secondary symptoms characteristic of blackleg—namely, yellowing and rolling of the leaves. There was no blackening of the stem above ground. The underground portions, however, showed brown lesions and a brown rot of the stem at the point of attachment to the seed piece and somewhat above it. None of the remaining plants showed symptoms of the disease. At digging time, on September 15, no decay of the tubers was found, with the exception of one very small tuber of the Netted Gem variety which showed a soft bacterial decay at the stem end. The progress of the decay, however, was checked, and the affected portion fell off, leaving only the sound part, so that the recovery of the causal organism was not possible.

Since the hot weather after May 5 might have had something to do with the slight progress of the disease in 1919, two sets of plantings were made on another piece of ground on the same farm in 1920—one on April 8 and the other on May 6. Only Irish Cobblers were used this time. Twelve tubers were cut in halves through the inoculated wound so as to make 24 seed pieces for each of the two series. Inoculations were made a few days before planting. Eight tubers were inoculated with the three western strains of the blackleg organism and 4 with the "B. sol." strain received from Dr. Morse. Up to July 20 four hills out of 16 inoculated with the western strains in the earlier planting and 2 out of 8 inoculated with "B. sol." in the same series showed typical field symptoms of blackleg, including an intense blackening of the base of the stem. On the other hand, no hill of the series inoculated with the same organisms and on the same plan, but planted one month later, showed any signs of infection. At harvesting time, on July 20, a number of tubers in the planting of April 8 showed blackleg-rot, and in the later planting only 2 tubers were found showing the same decay. It appears, therefore, that the earlier planting, when the soil and the air temperatures were

lower and the soil moisture was more abundant, greatly facilitated the development of blackleg.

FIELD OBSERVATIONS

The typical case of blackleg-rot on round varieties in the East has been figured in publications and charts issued by several agricultural institutions. As a rule, decay begins at the stolon end of the tuber with a comparatively small amount of rot visible on the outside or often only a small, black, circular opening. This opening leads to the interior of the tuber, where a progressive decay develops in the form of an irregular black, soft, or slimy hollow until nearly all of the tuber is consumed (Pl. 15, D, E). However, the development of the disease may deviate from this type even in eastern and northern sections of the United States, when conditions are abnormal and favorable to the disease, such as those in moist places or in wet seasons. Morse stated (*4*) with reference to blackleg in Maine that—

> When this disease occurs on a field it doubtless is responsible for much of the soft rot of the tubers observed in wet seasons.

It appears from certain observations made by the writers that under conditions of excessive soil moisture the bacteria in stems or seed tubers may be carried at least to the adjoining tubers of the same hill. The latter then become infected from the outside, or, if they are already infected through the stolons, the infection spreads in moist surroundings more rapidly on the outside over the surface of the tuber, or evenly throughout the flesh. Specimens of this sort were observed on the Eastern Shore of Maryland and Virginia (Pl. 15, G) and in Wisconsin, Minnesota, and Washington (Pl. 15, F) on various round types of potatoes. More accentuated symptoms of this order were found in irrigated sections of Colorado. The most peculiar manifestations of the blackleg tuber-rot were seen in the Snake River Valley of Idaho, where the Netted Gem variety is grown on a large scale. The following forms were observed there during a field survey arranged by the Office of Cotton, Truck, and Forage Crop Disease Investigations in 1920.

1. The stem-end rot of pointed-end Netted Gems. The external appearance of this form is extremely misleading (Pl. A, 1–4). It becomes prevalent in southeastern and eastern Idaho during the latter part of the season, shortly before the harvest. The relatively low temperature prevailing at this time of year is, no doubt, an important factor in the rapid progress of the disease. If the soil has plenty of moisture, freshly dug affected tubers show no shrinkage and preserve their natural shape (Pl. 15, A–C.). In the course of two weeks the shrinkage is evident and the decay takes on an inward trend (Pl. A, 4). By another two weeks, drying and folding of the decayed tissues become very pronounced, and the external appearance at this stage of the decay may well pass as an

illustration of any of a number of stem-end tuber-rots (Pl. 16, A, B). When a Netted Gem tuber of pointed shape affected with this type of the disease is cut open longitudinally as soon as it is removed from the ground, four distinct regions of decay may, as the rule, be seen: (*a*) The extreme stem-end region is usually decayed throughout; it is mushy or slimy in consistency and dark brown to black in color; in the field this seldom extends deeper than the outer demarkation line of the decay, but in storage under favorable conditions the disintegration advances more rapidly in the inner tissues of the tuber, taking on a cup-like shape and leading ultimately to the formation of a slimy cavity (Pl. A, 4; 14, B, D; 16, B). (*b*) An area of fresh decay appears within the core just beneath the first region; it is practically colorless, though it occasionally contains dark or black streaks, and in the very early stages it has the consistency of hardened butter (Pl. A, 4). (*c*) The cambium layer shows a brown discoloration extending sometimes close to the eye end; in advanced stages a portion of this region nearest to the stem end is more or less disintegrated and forms a channel attentuating toward the eye end until it gradually transforms into a mere browning of the vascular network which also gradually loses its intensity and finally disappears altogether; this condition is very distinct with some freshly dug tubers, but later on with the inward progress of the decay it becomes less pronounced (Pl. A, 1, 4; 16, B). (*d*) The decay of the outer layer develops in the bark region, is soft but not mushy in consistency and more or less dark-brown in color; it frequently extends over the tuber much farther than the pith decay, but not always as far as the cambium discoloration; its progress is checked after tubers are dug and exposed to drying (Pl. A, 1, 2; 16, B).

2. The shallow stem-end rot of round-shaped tubers. This type was observed mostly on the Idaho Rurals. Under conditions of abundant moisture the bacterial infection spreads from the stem end over the surface of the tuber and penetrates into the bark region, though not very deeply. When such tubers are taken out of the ground and exposed to the sun, as happens at digging, the infected areas dry up very promptly and form hard, black, shallow patches (Pl. 16, E). The condition may easily be mistaken for the black fieldrot described by Pratt (*6*) and attributed to *Fusarium radicicola*. If, however, the tubers are again transferred to a moist place with a moderate temperature, a soft, mushy bacterial decay is likely to develop beneath these dry areas. On the contrary, under conditions unfavorable to the blackleg decay the status may either remain unchanged or become complicated by the entrance of various Fusaria and other rot-producing fungi. In the latter case it is impossible to determine the original cause of the disease.

3. Siderot of either round or long potatoes. It may penetrate inside of the tuber to a considerable depth, and when a freshly dug diseased

tuber is cut open it reveals a colorless buttery or mushy decay with black streaks, usually on the border line of the diseased and healthy tissues. If exposed to drying the decayed areas may become spongy and very much resemble the texture which is usually observed in Fusarium rots (Pl. 16, D). In many instances, however, if the decayed region is sufficiently deep to prevent complete drying, sections through such tubers may show an inner layer of active bacterial decay. As is the case with the other forms of blackleg-rot this form, too, may become further invaded with various rot-producing or saprophytic fungi. The writers had under their observation a tuber of this type with a copious growth of Rhizoctonia on the outside all over the diseased area, while soft bacterial decay was still progressing within the tuber even in the dry laboratory atmosphere (Pl. 16, C).

SUMMARY

(1) An organism isolated from western stem-end rotting potatoes is identical with *Bacillus phytophthorus* Appel in all the essential characters commonly considered in the determination of bacterial species.

(2) It is pathogenic to the potato, and inoculations of healthy stems or tubers with pure cultures produce, respectively, a rapid, soft decay of stems or a tuber-rot.

(3) Blackleg tuber-rot under the field conditions in certain irrigated sections of the West, particularly in pointed-end Netted Gems, takes on a form atypical of the familiar manifestation of this disease in the East.

(4) The external appearance becomes especially confusing when the affected areas dry up and shrivel in storage, but usually the trouble may be identified by cultural work or by planting diseased tubers under control conditions.

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PLATE A

Types of blackleg potato tuber-rot on pointed-end Netted Gem from Idaho, showing external as well as internal appearance. The photograph was taken two weeks after the tubers were removed from the ground. The same tubers are shown in Plate 15, A, C, on the first day after digging.

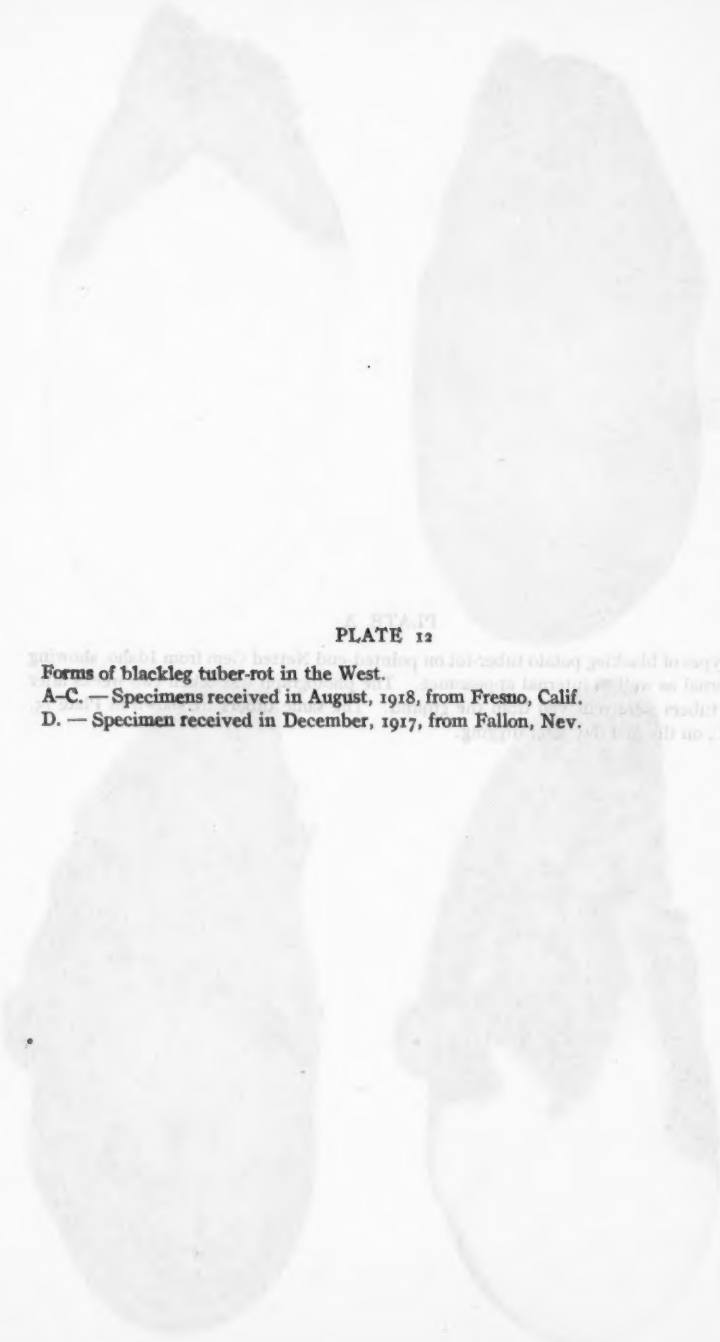

PLATE 12

Forms of blackleg tuber-rot in the West.
A–C. — Specimens received in August, 1918, from Fresno, Calif.
D. — Specimen received in December, 1917, from Fallon, Nev.

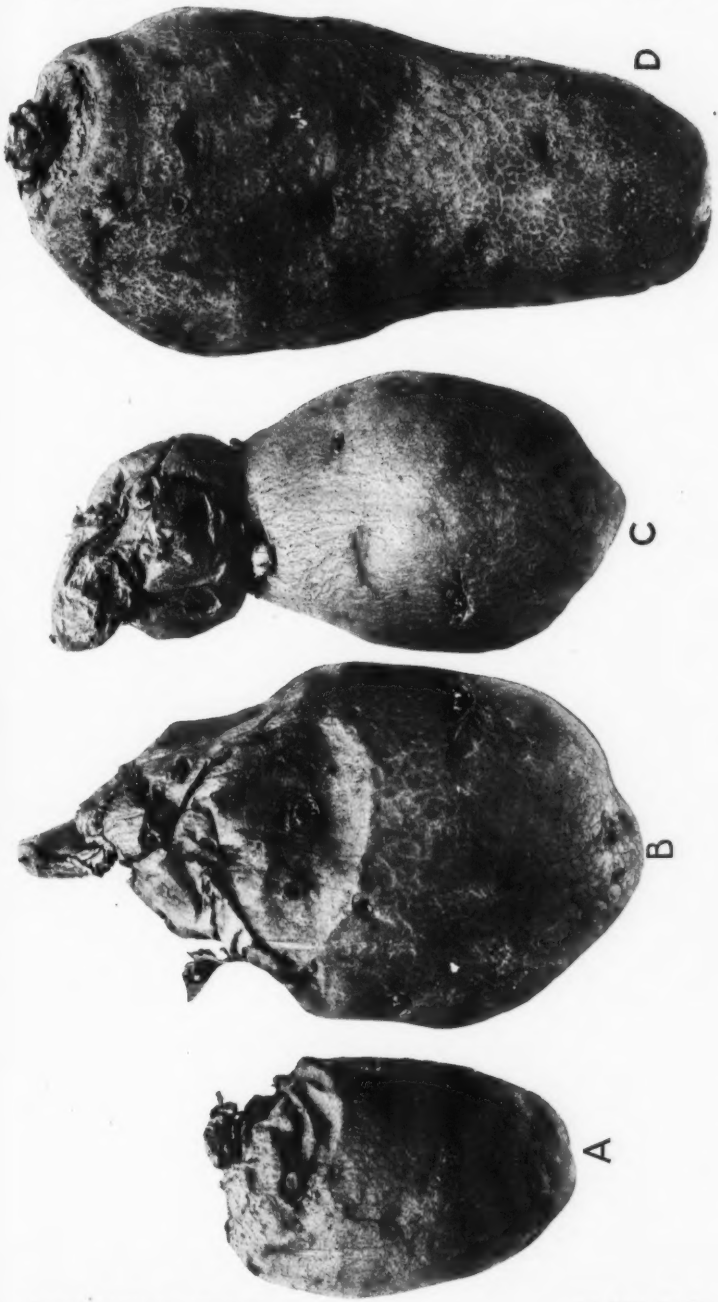
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PLATE 13

Blackleg on stems resulting from planting the diseased western material.
A.—Appearance of plant above the ground.
B.—Spread of the infecion from the diseased seed piece to the new stem.

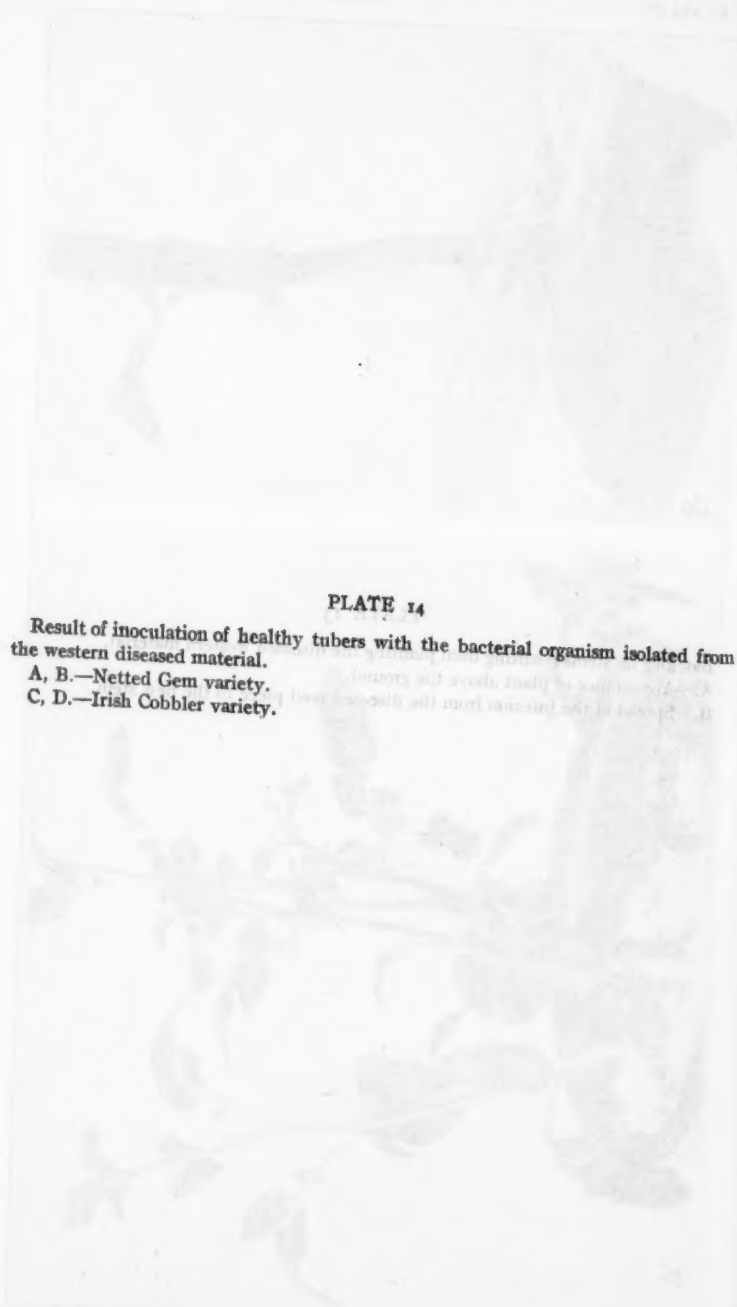
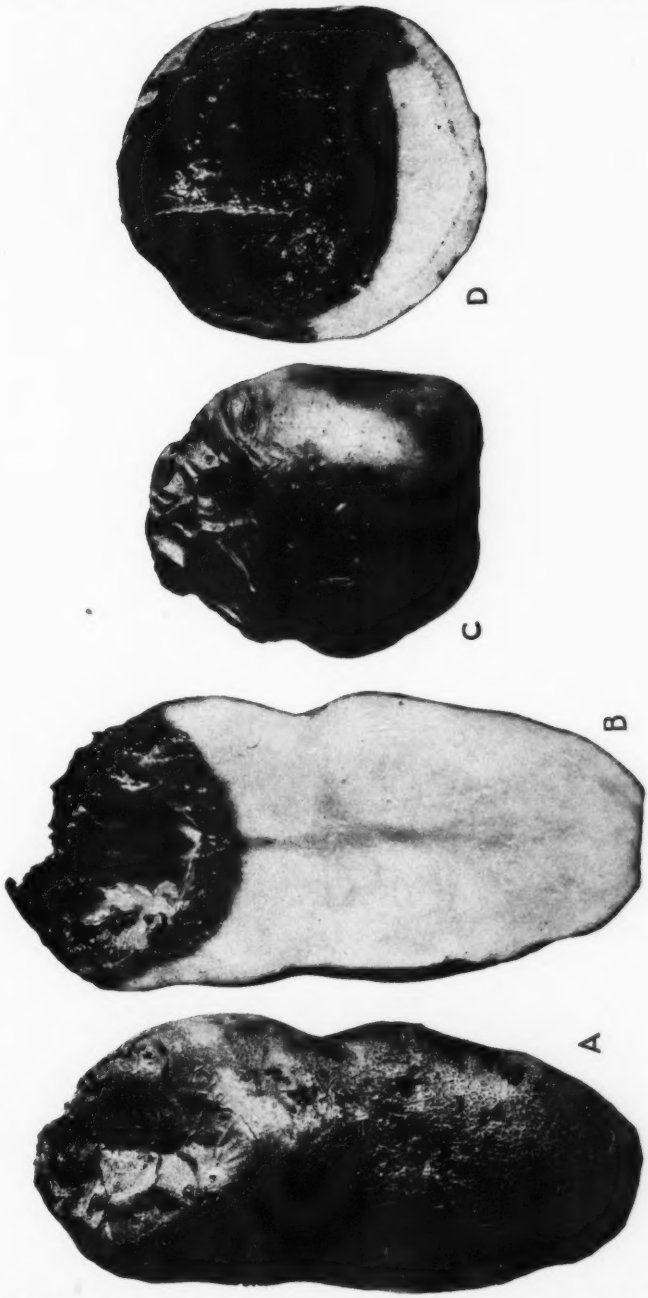


PLATE 14

Result of inoculation of healthy tubers with the bacterial organism isolated from the western diseased material.

A, B.—Netted Gem variety.

C, D.—Irish Cobbler variety.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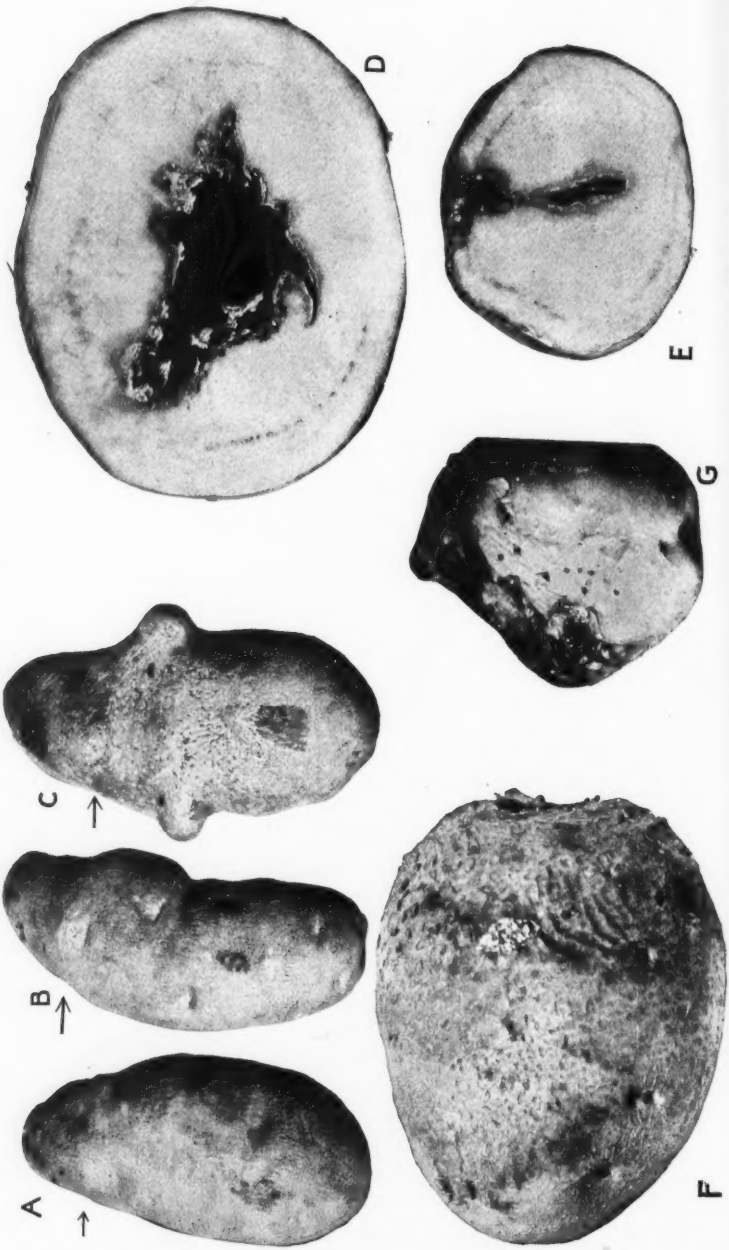
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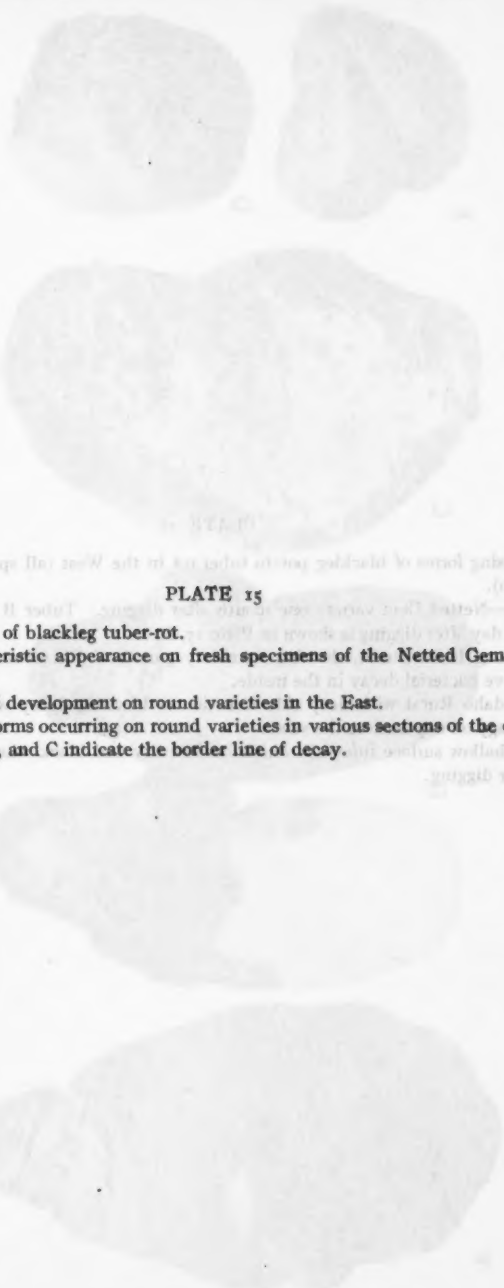


PLATE 15

Different types of blackleg tuber-rot.

A–C.—Characteristic appearance on fresh specimens of the Netted Gem variety from Idaho.

D, E.—Typical development on round varieties in the East.

F, G.—Other forms occurring on round varieties in various sections of the country.

Arrows in A, B, and C indicate the border line of decay.

PLATE 16

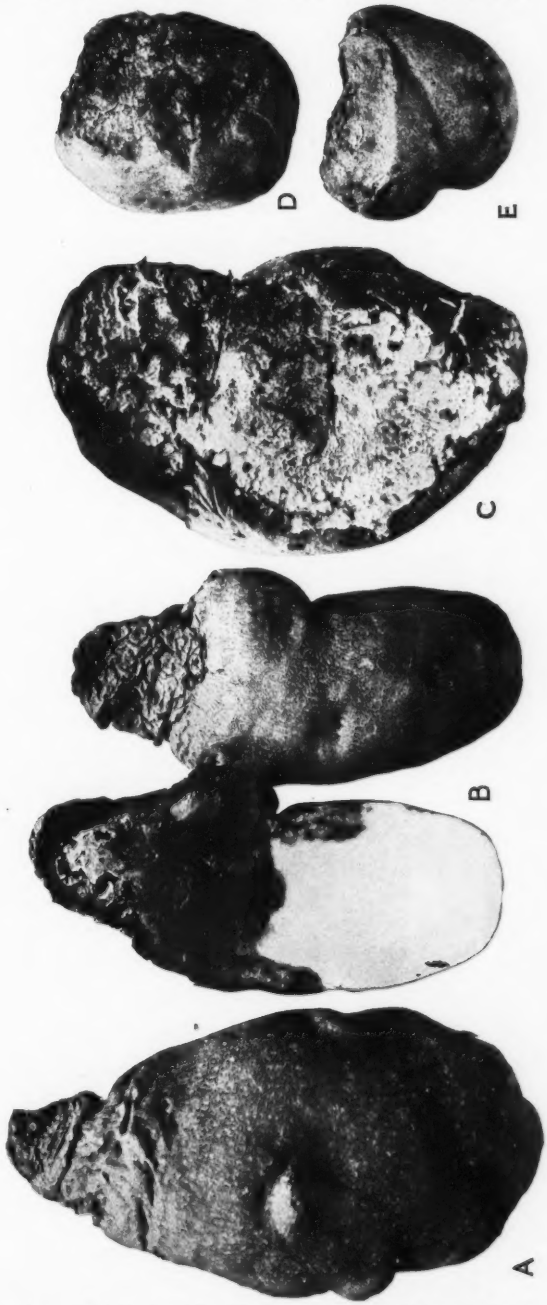
Confusing forms of blackleg potato tuber-rot in the West (all specimens collected in Idaho).

A, B.—Netted Gem variety one month after digging. Tuber B as it appeared on the first day after digging is shown in Plate 15, B.

C.—Long Idaho Rural, showing secondary growth of Rhizoctonia on the outside and active bacterial decay in the inside.

D.—Idaho Rural with deep side infection of blackleg decay which became dry and spongy on exposure to the sun.

E.—Shallow surface infection which became dry and black when exposed to the sun after digging.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MICROSCOPIC STUDY OF BACTERIA IN CHEESE

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INTRODUCTION

Heretofore cultural methods have ordinarily been used in the study of cheese flora, but the usual routine technic has given only an inadequate conception of the number and types of bacteria present. Although these cultural methods were employed in order to secure an idea of the number and varieties of organisms occurring in cheese, they have failed to establish the relative abundance of each type of microorganism in the cheese. This objection remains valid in the light of both quantitative and qualitative studies.

The so-called "dilution technic," which involves the mass action of the organisms, has proved valuable in determining the type or group of organisms which predominates in a given sample; but it does not furnish information regarding the general flora. This method often tends to give erroneous results, especially when the dilution medium used favors the growth of special groups. Under such conditions the results are influenced by the selective action of the medium. This has been true where milk was used as a dilution medium in examining cheese. The milk favored the growth of the lactic acid group; while the inert and nonlactose fermenting types or slow-growing cocci were overgrown, due in a large measure to the selective action of the medium. This procedure has well served its purpose in assisting to isolate the organisms for which it has a special adaptation.

In general, cultural methods are preferable to a microscopic examination because cultures can be isolated and studied independently—a feature which will always remain the outstanding advantage of these methods.

Although subject to the same limitations as any microscopic method, the following method has been successfully used in this laboratory and has been employed in a routine way in determining the number of bacteria in cheese.

HISTORY

Johan-Olsen (*8*)[1], working with the molds which ripen "Gammelost" (a Norwegian cheese), mentions a sectioning method and implies that it resembles the usual histological technic but does not outline the procedure in detail.

[1] Reference is made by number (italic) to "Literature cited," p 100.

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Troili-Peterson (*11*), in discussing the bacterial flora of Swedish "Gûterkäse," mentions the microscopic examination of cheese as a control for the cultural procedure but does not give the technical details. She presents photomicrographs of cheese sections and states that some of the preparations were stained in methylene blue and that a few were examined unstained.

Gorini (*3*), in studying the distribution of the bacteria in Grana cheese, presents the details of a method by which he prepared sections for microscopic examination. In his procedure he fixed and dehydrated samples of cheese by passing them through a series of alcohols of increasing concentrations until a strength of 95 per cent was reached. The usual histological methods of sectioning were followed, and the sections were stained in an aqueous solution of methylene blue.

In the following year Rodella (*9*) reported a method used in his laboratory for preparing sections used in the direct examination of cheese samples. With his technic the samples were dehydrated and fixed by a method similar to that of Gorini and sectioned in the usual way. He found, however, that carbol-thionin gave better results as a stain than did methylene blue.

Harrison (*6*) outlined in detail a method for embedding and sectioning cheese which is similar to the common histological method, but like his predecessors he made no estimate of the number of bacteria present.

During the year in which Rodella (*9*) presented his paper, Troili-Peterson (*12*) and Gorini (*4*) published notes discussing the question of priority raised by the practically simultaneous publication of their papers. It appears that the methods followed by Trioli-Peterson were similar to those of Gorini, but that she did not feel the necessity of presenting the technical details because of the universal knowledge of the common embedding methods.

No results have been obtained in any of this work that permit a comparison between counts made by the plate method, so commonly used in floral studies of cheese, and counts made by direct microscopic examination. Following the method outlined below, comparatively accurate counts have been made by the direct method, and the number of the different types of bacteria have been determined as they actually exist in the cheese mass.

TECHNIC

EMBEDDING AND SECTIONING

The samples of cheese were embedded by the usual histological technic and sectioned with a Minot rotary microtome. In sectioning, the microtome was so adjusted as to give sections 5 μ thick. The sections were stained by the Gram method and with an aqueous solution of methylene blue.

In order to determine the effect of the embedding process upon the cheese, small measured cubes of cheese were subjected to the routine procedures. Only a slight shrinkage was found, indicating that the volume of embedded cheese when examined is approximately the same as that of the fresh sample.

MICROSCOPIC EXAMINATION

The preparations were examined with an oil immersion lens and a high power ocular, the most satisfactory combination being a 1.9-mm. fluorite objective with a numerical aperture of 1.32. Where a thick coverslip was used it was necessary to have a 3-mm. apochromatic objective with a numerical aperture of 1.4. Greater depth can be secured with compensating oculars than with the ordinary Huygenian oculars.

The method, although at first used only for determining the types of organisms present in the samples and as a check on the usual plate method, was found useful as a means of determining the number of organisms present. In order to make such a computation the microscope was so standardized as to allow an estimate of the number of organisms per gram when only a small amount of the original section was examined. This computation is similar to that used in the direct method of counting bacteria in milk described by Breed and Brew (*2*). This was accomplished by measuring both the diameter of the microscopic field and the thickness of the section from which the amount of cheese actually seen in each field examined was determined. Knowing the volume and specific gravity of the cheese examined, the total number of organisms per gram can readily be computed. With the diameter of the field measuring 0.14 mm. (140 μ), the microtome so adjusted as to cut sections of a thickness of 0.005 mm. (5 μ), and a specific gravity of 1, the amount of cheese examined per microscopic field would be 1/13,000,000 gm.—that is, each organism observed in a single microscopic field represents 13,000,000 per gram.

This factor may be computed by the following formula, in which any measure may be substituted:

$$\frac{1{,}000}{\pi r^2 a} b = \text{factor per gram.}$$

In the above formula,

r = the radius of the field examined in millimeters as determined by actual measurement.

a = the thickness of the section in millimeters.

b = the specific gravity of the cheese.

The radius of the field, as has been stated, is determined by measurement with a stage micrometer and varies with the magnification and with the type of ocular used. However, it was found advisable to adjust the

draw tube of the microscope so that the field would be of the greatest possible diameter without losing definition, as the greater the diameter of the field the less the increment of error in the total counts.

The thickness of the section is controlled by adjusting the microtome to cut sections of a desired and known thickness. If all the adjustments on the microtome are firm and a sharp knife is used, sections can be cut of uniform thickness with surprising accuracy. The thickness of the sections can also be remeasured with the fine adjustment screw on the microscope. Although not perfect, this method of measurement serves as a check upon the accuracy of the sectioning. The measurement is accomplished by focusing with the graduated fine adjustment screw on both the upper and lower surfaces of the section and noting the differences in the readings between the two levels. The difference can be read in microns where graduations are given on the fine adjustment screw.

To convert the per-cubic-centimeter counts into numbers per gram, the specific gravity of the cheese must be considered. As the specific gravity of all samples has been assumed to be approximately 1, the counts are interchangeable. This assumption in regard to the specific gravity is arbitrary, but the variations in the specific gravity of cheddar cheese are so slight that the total count is not affected to any appreciable degree. Accurate determinations did not seem practicable, as the specific gravity varies with the fat content and with the moisture and general consistency of the cheese.

With the measurements and adjustments used in this laboratory the per-gram formula resolves itself into the following:

$r = 0.07$ mm. (70 μ).

$a = .005$ mm. (5 μ).

$b = 1.0$.

$$\frac{1{,}000}{3.1416 \times 0.0049 \times 0.005} \times 1 = \text{approximately } 13{,}000{,}000.$$

APPLICATION OF THE METHOD

It is evident that this microscopic technic is subject to the limitations of any direct method of examination, many of which are unavoidable and are due to mechanical limitations or to the human error, which enters in when counts or estimates are made.

QUALITATIVE EXAMINATION

As previously stated, cheese has been examined microscopically by many investigators. The possibility of error is not as great when samples are examined to determine the types of organisms present as when total count is made, which is true of any microscopic work. Our present staining methods make possible a direct visualization of the microorgan-

isms together with their morphological and other general characteristics, but an attempt to enumerate these types involves other difficulties.

The direct examination of cheese in the different ripening stages is advantageous and important, since the different groups of organisms can be studied as they actually occur in the cheese mass, and their groupings and relative relationships noted. The grouping may be especially important when considered in relation to the number present. For example, an organism may be present in large numbers during the early stages of ripening, but appear in scattered and isolated groups containing only a few individuals. In some instances only single bacteria were found through the mass. In such cases the total number of this group by the plate count may be large, but the grouping, as determined by direct examination, may demonstrate that they are not actively growing and playing a part in the ripening of the cheese. On the other hand, the presence of large clumps of organisms, with the size of the clumps increasing during ripening, indicates that such groups are developing in the cheese mass and are probably playing an important rôle in the changes involved.

That this grouping of the organisms actually occurs can be seen in Plate 17, A. In this photomicrograph are shown the types and groupings of organisms found in a very green cheese, showing that the *Streptococcus lactis*-like organisms predominated and were scattered in pairs over the field. Any migration of these bacteria through the cheese mass appears to have been impossible, and one is impressed with the fact that growth and reproduction could not have been taking place rapidly or the number of individuals per group would have been larger. In Plate 17, B, which represents a section from a cheese 5 months older than that shown in Plate 17, A, the organisms are found in larger clumps with many of them so massed that accurate counting is impossible. From the examination of a series of sections from cheeses of varying ages, it has been found that the clumps increase in size as the cheese ripens, reaching a limit after seven to eight months. It is evident that the organisms in the clumps, mostly cocci and a few rods, are thriving and reproducing and must, therefore, change the surrounding medium as they utilize it for food. It is not within the scope of this paper to discuss the significance of this occurrence but only to point out that such variations are found when samples are examined directly.

QUANTITATIVE EXAMINATION

An objection often made to counting organisms in microscopic preparations of dried liquids is the uneven thickness of the resultant dried film. This objection is eliminated when paraffin sections are used, as such sections are uniform in thickness and the organisms remain in their natural relationships. Boekhout and DeVries (*1*) at one time endeavored

to show that the scattered organisms in cheese sections were due, in a large measure, to the breaking up and scattering of the clumps by the knife edge. This explanation will hardly appear plausible to anyone familiar with the perfection of delicate histological sections prepared with a sharp knife.

The grouping and clumping of the organisms often cause difficulty in accurately determining the number of organisms in the cheese sections. This is especially true in sections of old cheeses in which the bacteria tend to clump in large masses. The error can be overcome to a large degree by counting or estimating a large number of fields, the larger the number examined the smaller being the error in the final estimate.

In a sample of green cheese where the organisms appear in large numbers, but are evenly scattered, it is impracticable to count the entire field, and an ocular disk divided into quadrants may be inserted in order to facilitate accurate counting.

In all cases 20 or more fields should be counted, and especially where the organisms are unevenly distributed. In such instances, typical fields which represent the general flora should be located by studying the entire section.

COMPARISON OF DIRECT AND PLATE COUNTS

Table I gives a few representative comparisons between direct microscopic and plate counts made from cheese samples in various stages of ripening. The plate counts average approximately one-twelfth the direct count, but no common ratio has been found to exist between the results obtained by the two methods. Wide variations in the ratios between the counts were found, but in general the ratios from green cheese appeared to be larger than those from cheese more advanced in ripening.

The above plate counts compare well with those found by other observers who have examined cheddar cheese, Russell (*10*) found from 62 to 665 million per gram. Harrison and McConnell (*7*) found the count to be as high as 625 million per gram in the earlier stages of the ripening, while Harding and Prucha (*5*) observed from 37 to 177 million per gram.

Several explanations may be offered to account for the apparent discrepancy between the results obtained by the two methods. The plate count is an estimate based on observations of the growth of organisms on some particular medium which, in cheese investigations, usually contains lactose. Lactose has been generally used because media containing this particular carbohydrate have been found to allow the development of a larger number of colonies than do sugar-free media. Investigators have based their cultural methods upon media giving the largest counts rather than upon media which might serve as an index to the relative number of types present. In comparing the microscopic counts with results obtained with the plate method, it may be noted that the types

present in the cheese, as seen by direct examination, are not present in the same proportions on the plates, because those types which grow abundantly in the presence of lactose have outnumbered all groups which do not grow as readily on such media.

TABLE I.—*Relation between microscopic and plate counts obtained from cheese*

Cheese.	Approximate age of cheese.	Miscroscopic count (millions per gram).					Plate count (millions per gram).		
		Cocci.	Short rods.	Yeast.	*Streptococcus lactis.* (Lister)	Total.	*Streptococcus lactis.*	Miscellaneous.	Total.
OOCII XII 2	2 months	100	600	.....	4,500	5,200	46	9	55
OOCI$_3$ XII 2	2 months	14,650	5,200	.....	21,450	41,300	264	284	548
6, 4, II	6 months	312	.....	26	143	481	132	90	222
B C II	3 months	1,690	780	.....	6,760	9,230	251	41	292
6, 26, II	5 months, 23 days	962	260	.....	988	2,210	306	121	427
OO$_3$8I8	Unknown; appeared green.	338	26	.....	3,029	3,393	.....	.....	702

Results of plate counting may also be lower because of insufficient grinding and emulsifying of the cheese sample previous to plating. This appears to be especially significant in cases where investigators grind the sample with sterile quartz or sugar and suspend the ground mass in sterile water in preparation for plating. If the sample is not well ground, small particles of cheese remain in the emulsion, and the individual bacteria are not separated so as to allow them to grow into separate colonies on the artificial medium. Emulsions examined under the microscope often show comparatively large masses of cheese which have not been affected by the grinding process.

CONCLUSION

The microscopic examination of cheese embedded and sectioned by the usual histological method is a valuable and satisfactory method for studying the different stages of cheese ripening. Such a direct method of examination may be used to determine the number of organisms present in the sample. It also serves as an index to the types of organisms present and makes possible a study of the organisms as they actually exist in the cheese mass, allowing observations on the groupings and relationships during cheese ripening.

The cultural methods do not yield as high a count as the microscopic method, due primarily to the selective action of the medium used and the difficulty of liberating the organisms from the cheese mass previous to plating.

A combination of microscopic and cultural studies yields a far more complete picture of what takes place in cheese ripening than can be obtained by the use of either method alone.

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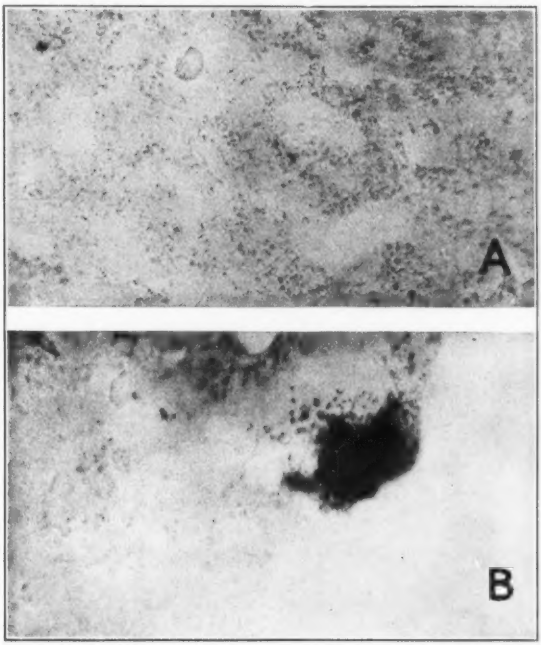
PLATE 17.

A.—Section of cheddar cheese 1 month old, stained with an aqueous solution of methylene blue, showing isolated pairs of *Streptococcus lactis* Lister throughout the field. × 500.

B.—Section of cheddar cheese 6 months old, stained as in A. × 800.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B

FURTHER STUDIES ON RELATION OF SULPHATES TO PLANT GROWTH AND COMPOSITION

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This is a continuation of an investigation, part of the results of which were reported in a former publication (*4*).[1] As stated in the earlier paper, the addition of the different forms of sulphur caused a marked increase in the dry weight of red clover, and beneficial results were obtained with oats and rape. One very noticeable result observed in the former work was the high nitrogen content of the clover grown on soils in the greenhouse receiving sulphur fertilizer, compared to that of clover receiving only the residual sulphur of the soil.

This great increase in nitrogen assimilation by the clover where sulphates were applied, and under the conditions described, led the writer to believe that the sulphates favorably influenced the activity of the legume bacteria. Especially did this appear true where the beaverdam soil was used. This soil contained 0.18 per cent sulphur with appreciable quantities of sulphate sulphur in the soil extract, and no beneficial result from sulphur fertilizer was expected. Oats did not respond to sulphur with this soil, although the sulphur content of oats (*3*) and the amount of sulphur removed by one crop of oats is as large as with a red clover crop. From present data, the responses of red clover so often obtained with gypsum compared to cereals can not be explained through a difference in sulphur requirement. With alfalfa the amount of sulphur removed is so large compared to the cereals and red clover that the addition of sulphates would apparently function directly as a plant food where increased growth results. An example of the latter would be the enormous increases in the yield of alfalfa obtained in southern Oregon (*7*) where sulphur fertilizers were applied to soils with a very low sulphur content. These authors, however, mention the favorable action that sulphur fertilizers had on the root development and nodule production of alfalfa. Duley (*2*) reports increase nodule production on red clover where sulphur was added to soils. Pitz (*5*) observed increased nodule production and root development with red clover by applying gypsum to soil cultures.

As far as the writer can ascertain, no correlation has been shown between nodule production and nitrogen content of the plant, by influencing the development of the former, with ordinary sulphur fertilizer compounds. In this paper a study has been made of the effect of different

[1] Reference is made by number (italic) to "Literature cited," p. 110.

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concentration of sulphates on growth and nitrogen assimilation, and also the relation of total sulphur content of the plant as influenced by available nitrogen. The red clover and rape were used in this work. With clover, the initial concentration of legume bacteria has been varied by inoculating certain cultures, while others were not inoculated.

TABLE I.—*Analytical results with red clover on Medford loam soil*

Treatment.	Weight of air-dried clover.	Total N.	N insoluble in acetic acid.	Total S.	Sulphate S.	Organic S.	S in acetic-acid solution.	S insoluble in acetic-acid solution.	Weight of air-dried roots.	Total S in roots.	Total N in roots.	Ratio of tops to roots.
Control:	*Gm.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*Gm.*	*P. ct.*	*P. ct.*	
Uninoculated..	15.84	2.88	2.13	0.20	0.06	0.14	0.10	0.10	6.00	0.32	1.69	2.64
Inoculated.....	21.24	3.31	2.10	.20	.04	.16	.08	.12	7.65	.34	2.16	2.77
$NaNO_3$:												
Uninoculated..	17.70	2.81	2.02	.18	.07	.11	.08	.10	6.45	.28	1.64	2.74
Inoculated.....	21.29	3.31	2.10	.16	.02	.14	.07	.09	7.80	.23	1.83	2.73
$NaNO_3$ and Na_2SO_4:												
Uninoculated..	18.82	3.30	2.15	.26	.10	.16	.15	.11	7.60	.60	1.91	2.48
Inoculated.....	23.35	3.36	2.22	.28	.12	.16	.17	.11	7.10	.72	1.91	3.29
Na_2SO_4:												
Uninoculated..	15.00	3.58	2.31	.40	.25	.15	.31	.09	8.60	.59	1.73	1.74
Inoculated.....	20.31	3.48	2.19	.34	.14	.20	.20	.14	8.20	.53	2.17	2.47
$CaSO_4$:												
Uninoculated..	16.49	3.46	2.39	.28	.13	.15	.15	.13	7.20	.50	2.04	2.29
Inoculated.....	16.65	3.38	2.30	.34	.15	.19	.20	.14	5.70	.61	2.05	2.92
$NaNO_3$ and $CaSO_4$:												
Uninoculated..	14.18	3.33	2.28	.41	.27	.14	.28	.13	6.10	.55	1.75	2.32
Inoculated.....	16.89	3.34	2.33	.33	.15	.18	.18	.15	6.20	.55	2.00	2.72

In the first experiment Medford loam soil, designated as soil B in the previous publication (*4*), was used. This soil was heated in an electric oven where the temperature was gradually raised to 120° C. and maintained for six hours. This was to destroy the legume bacteria present in the soil. Four kgm. of soil, after being mixed with the different fertilizers, were placed in paraffined clay pots and carefully seeded to red clover. One series was inoculated with *Bacillus radicicola* Bey. The solution for inoculation was prepared by removing the growth of organisms from a culture and mixing with water. Each inoculated soil culture received a definite number of cubic centimeters of the bacterial solution, concentrated where the seeds were placed. The amount of different fertilizers added per pot was as follows: Sodium sulphate (Na_2SO_4), 3 gm.; sodium nitrate ($NaNO_3$), 2 gm.; calcium sulphate ($CaSO_4.2\ H_2O$), 3.75 gm.; and calcium carbonate ($CaCO_3$), 3 gm. The cultures were placed in the greenhouse on October 16 and harvested on March 18. Ten plants were allowed to grow in each pot. The cool temperature in the greenhouse did not permit rapid growth, and the plants were cut before maturity was reached. The treatment and analytical results are given in Table I. The cultures were weighed every other day, and the moisture was maintained at 20 per cent. In removing the roots the soil was shaken out of the pot and carefully loosened. The roots were then separated out and washed. After drying they were weighed, and the non-

volatile matter was determined by ashing a ground sample representing each culture. This was done to correct for any excess weight due to adhering soil particles.

The total sulphur was determined by the sodium-peroxid method. The sulphate sulphur was extracted by taking 2 gm. clover and 150 cc. of water and digesting on the steam bath for three hours. It was then slightly acidified with hydrochloric acid, and after standing for an hour the extract was filtered. Five cc. of 10 per cent barium-chlorid solution was used to precipitate the sulphate sulphur in the hot solution. After standing overnight the barium sulphate settled to the bottom of the beaker in all cases, and no particles of precipitate could be detected in the supernatant liquid. This liquid was carefully decanted off; and the white precipitate was washed on a Gooch crucible, dried, and weighed. Several of these precipitates were ignited, but since no appreciable loss in weight was detected this method appeared perfectly reliable for comparable results on sulphate sulphur in the different samples of plant material. For the determination of total nitrogen insoluble in acetic acid, 1-gm. samples were digested with about 150 cc. of water on the water bath for two hours. The extract was then acidified with dilute acetic acid and filtered after standing about 30 minutes. The total nitrogen was determined on the precipitate by the Kjeldahl method. The filtrate was made alkaline with sodium carbonate, evaporated to a few cubic centimeters in volume, transferred to a nickel crucible, and total sulphur determined by the sodium-peroxid method.

The dry weights of plant material produced show no increase in production that can be attributed to presence of sulphates. This result is different compared to the noticeable increase reported with the same soil before (*4*). As mentioned above, conditions were very unsatisfactory for growth, and the plants were cut before maturity five months after planting. In the former work reported, conditions permitted rapid growth, and the plants, though not mature, were harvested two months after seeding. As the soil used in this later work had been heated, there was perhaps some change in degree of solubility of soil minerals and in the biological flora. The concentration of added mineral salts was also greater in this work.

Examination of the roots showed that all plants had become infected. In the uninoculated series, roots from cultures 1 and 2 contained very few nodules compared to the roots grown in the soil receiving sulphate fertilizer. This remarkable difference in nodule formation no doubt accounts for the low nitrogen content of the clover plants in pots 1 and 2. That these plants became infected without any artificial inoculation is not surprising. Wilson (8) found that—

> of fifteen legumes grown in Volusia silt loam soil, only one, Trifolium pratense, developed nodules without artificial inoculation.

During the growth of the plants the inoculated series showed greater development, which is apparent upon examining the dry weights. Cultures 1 and 2 of the inoculated series show no effects from lack of sulphates, and all the sulphur-fertilized pots contained numerous well-developed nodules.

The total nitrogen insoluble in acetic acid was no greater in some of the cultures receiving added sulphates than in the controls, so no statement can be made that sulphate addition caused this fraction to become larger. The nitrogen content of the fraction soluble in acetic acid is lower in 1 and 2 of the uninoculated series. The increase in percentage of sulphur caused by fertilizer treatment is accounted for generally by higher sulphate content. Although the organic sulphur is apparently higher in some, the results are not consistent with the total sulphur, to state that the former results from increased sulphur assimilation in this experiment. Total sulphur in the acetic-acid extracts runs parallel with sulphate sulphur results and is slightly higher, showing that there is some sulphur in the organic form not accounted for in the precipitate from acetic-acid solution. This was also found to be true with clover grown in other pots which was harvested while in blossom.

The ratio in weight of tops to roots is greater in the inoculated sulphur-fertilized cultures than in the uninoculated sulphur-fertilized cultures. This difference does not appear to be due to inoculation alone, for this does not hold true in comparing 1 and 2 of both series, while the ratio of tops to roots in 1 and 2 of the uninoculated series is greater than the remaining four where there is heavy nodule growth. Arny and Thatcher (*1*) report a greater ratio in weight of tops to roots where inoculation was made with alfalfa and sweet clover.

The sulphur content of the roots is larger than in the other portion of the plant, whereas the opposite is true in percentage of nitrogen.

The second part of this work was carried on with beaverdam soil and red clover. Each pot contained 7 kgm. of soil, and the following amount of fertilizers were added as indicated in Table II: 12 gm. of calcium sulphate, 10 gm. of sodium sulphate, 2 gm. of sulphur, and 6 gm. of sodium nitrate. Two gm. of potassium chlorid and 10 gm. of calcium carbonate were added to all the soil cultures. Twenty red clover plants were allowed to grow in each pot, and the moisture content was kept at 40 per cent. The first crop grew at the same time as the clover on the Medford loam soil and was also cut before the blossoming stage. Three other crops were grown on these same cultures. The first was harvested on March 24, the second on May 20, the third on July 9, and the fourth on August 17. The last three crops were cut during the blossoming stage. The results are given in Table II.

TABLE II.—*Analytical results with red clover on beaverdam soil*

Treatment.	Crop I.			Crop II.			Crop III.				
	Weight of air-dried clover.	Total N.	Total S.	Weight of air-dried clover.	Total N.	Total S.	Weight of air-dried clover.	Total N.	Total S.	Sulphate S.	Organic S.
	Gm.	*P. ct.*	*P. ct.*	*Gm.*	*P. ct.*	*P. ct.*	*G.m*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Control	46	3.28	0.21	94	2.55	0.14	69	2.43	0.14	0.02	0.12
$CaSO_4$ and $NaNO_3$	43	3.50	.21	98	2.70	.20	66	2.67	.22	.....	.....
$CaSO_4$	46	3.41	.21	84	2.50	.20	52	2.67	.21	.06	.15
$NaNO_3$	53	3.25	.19	102	2.40	.14	57	2.39	.14	.02	.12
Na_2SO_4	44	3.47	.21	91	2.53	.16	52	2.72	.20	.07	.13
Na_2SO_4 and $NaNO_3$	45	3.12	.22	80	2.49	.20	58	2.78	.20	.....	.....
S	45	3.42	.22	83	2.47	.17	58	2.65	.21	.....	.....
S and $NaNO_3$	46	3.58	.23	88	2.56	.16	63	2.72	.21	.....	.....

Treatment.	Crop IV.				
	Weight of air-dried clover.	Total N.	Total S.	Sulphate S.	Organic S.
	Gm.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Control	27	2.88	0.03	0.03	0.15
$CaSO_4$ and $NaNO_3$	28	3.10	.30	.15	.15
$CaSO_4$	24	3.15	.27	.12	.15
$NaNO_3$	24	2.89	.22	.06	.16
Na_2SO_4	27	3.01	.27	.11	.16
Na_2SO_4 and $NaNO_3$	29	3.22	.29	.13	.16
S	29	3.16	.28	.14	.14
S and $NaNO_3$	26	3.10	.28	.12	.16

Examination of the foregoing data shows no result from sulphate application in the first two crops. There is no increase in dry weight in the pots receiving sulphur fertilizers, and in some crops the yield on the control is greater. In the third and fourth crops, one distinctive difference appears in the lower nitrogen content of the clover grown on the control soil cultures. The fact that the clover grown under conditions supplying more sulphate sulphur has a higher nitrogen content adds to the data already obtained pertaining to the favorable influence that sulphates have upon legume bacteria, the action of which results in a higher nitrogen content of the clover. According to experimental evidence, sulphates do not increase nodule production on all legumes. Wilson (*8*) reports that certain sulphates depressed nodule formation on the soybean. On the other hand, Prucha (*6*) mentions magnesium sulphate and calcium sulphate as exerting a beneficial influence on nodule development of the Canada field pea.

The low nitrogen content did not appear to be due to the absence of sulphates in the plant tissue, thus limiting protein synthesis, as sulphates were present in all samples. However, the percentage of sulphate sulphur was lower in clover grown on the control pots.

That the presence of available nitrogen or nitrogen assimilation by the plant tends to control or limit the total sulphur assimilation is illustrated by data in Table III.

In this experiment sea sand was used which had been washed with dilute hydrochloric acid and large volumes of distilled water. The sand still contained sulphur compounds, but no sulphates soluble in dilute hydrochloric acid. Six kgm. of sand were mixed with 10 gm. of calcium carbonate and placed in paraffined clay pots. Where elemental sulphur was used, 0.75 gm. was added at the same time. The other nutrients added were applied in solution form through a period of 70 days; the growing period was 80 days. The total amount of sodium sulphate which was added varied in the different cultures. Concentration 1 denotes 1.55 gm., concentration 2, 3.10 gm., etc. The same figures apply to calcium sulphate too. All cultures, with the exception of No. 3, 13, 20, and 21, received 3.9 gm. of sodium nitrate, and they each received 0.75 gm. Every culture received 2.6 gm. of potassium dihydrogen phosphate and 1.3 gm. of magnesium chlorid. Twenty plants grew in each pot, and the moisture content varied fron 15 to 20 per cent in the different cultures. The weights recorded are the average of duplicates, and the analyses were made on a sample obtained by mixing the duplicates. The 22 cultures from 11 to 21, inclusive, were inoculated with legume bacteria and 5 gm. of beaverdam soil added to the same culture.

TABLE III.—*Analytical results of clover grown on sand receiving a nutrient solution*

Uninoculated series.						Inoculated series.					
Culture No.	Form of sulphur added.	Sulphate concentration.	Weight of air-dry clover.	Total S.	Total N.	Culture No.	Weight of air-dry clover.	Total S.	Total N.	Sulphate S.	Organic S.
			Gm.	*Per ct.*	*Per ct.*		*Gm.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
1	Na_2SO_4	1	9.65	0.26	3.76	11	10.00	0.27	3.62	0.12	0.15
2	do	3	10.7	.42	3.77	12	7.00	.30	3.71	.12	.18
[a]3	do	1	3.95	.30	2.00	[a]13	4.80	.20	2.49	.08	.12
4	Na_2SO_4 and $CaSO_4$	2	8.75	.30	3.50	14	6.45	.32	3.70	.18	.14
5	Na_2SO_4	2	6.9	.34	3.69	15	6.85	.29	3.60	.15	.14
6	Control	.....	4.15	.35	3.90	16	7.3	.27	3.90	.08	.19
7	S	.....	6.62	.27	3.75	17	7.77	.32	3.69	.17	.15
8	$CaSO_4$	2	7.02	.34	3.85	18	7.30	.32	3.70	.18	.14
9	do	1	5.1	.34	3.80	19	5.90	.30	3.90	.....	.....
10	do	1	.....	.....	.....	[a]20	7.50	.20	2.42	.09	.11
11	Na_2SO_4 and $CaSO_4$	2	.....	.....	.....	[a]21	5.07	.26	2.46	.14	.12

[a] Low nitrate.

In the foregoing data the low sulphur contents occur in the clover grown in the pots receiving less nitrate nitrogen. In the inoculated series the sulphur content of the clover does not appear to increase by increasing the sulphate sulphur of the nutrient media. On the other hand, where there is a reduction in nitrate added, there is an appreciable reduction in the sulphur content of the clover. In the other series, where the concentration of legume organisms was not as great at the start, the percentages of sulphur generally run higher. In No. 3 the percentage of

sulphur is not lower than in some of the others, but here the total yield is small, and this often accounts for higher percentages of certain elements. The yields in No. 3 and 6 are about the same; but the sulphur content is higher in 6, although this culture depended only upon the sulphur in the sand. No. 2 shows response in sulphur content to the higher concentration of sulphates in the media. The corresponding culture 12 in the other series does not show higher sulphur content; and as the average sulphur content is lower in this uninoculated series, it appears that the legume organisms might have some effect on limiting the quantity of sulphur present in the clover hay.

TABLE IV.—*Data showing the sulphur-nitrogen relation in the portion insoluble in dilute acetic acid*

	In soil treated with—					Ratio of N to S in the insoluble portion.	Average ash content.
	$CaSO_4$ and $NaNO_3$.	$NaNO_3$.	Na_2SO_4.	Na_2SO_4 and $NaNO_3$.	Average.		
Crop I:							
Percentage of N insoluble in acetic acid	*Per ct.* 2.26	*Per ct.* 2.24	*Per ct.* 2.58	*Per ct.* 2.15	*Per ct.* 2.31		*Per ct.*
Percentage of S soluble in acetic acid	.08	.07	.09	.07	.....	17.7	9.94
Percentage of S insoluble in acetic acid	.13	.12	.12	.15	.13		
Crop II:							
Percentage of N insoluble in acetic acid	1.70	1.57	1.64	1.73	1.66		
Percentage of S soluble in acetic acid	.11	.06	.07	.11		18.8	7.31
Percentage of S insoluble in acetic acid	.09	.08	.09	.09	.088		
Crop III:							
Percentage of N insoluble in acetic acid	1.78	1.76	1.87	1.85	1.81		
Percentage of S soluble in acetic acid	.13	.05	.10	.11	.....	19.4	6.95
Percentage of S insoluble in acetic acid	.09	.09	.10	.09	.93		
Crop IV:							
Percentage of N insoluble in acetic acid	2.05	1.97	1.98	2.12	2.03		
Percentage of S soluble in acetic acid	.19	.09	.16	.18	.....	17.5	8.2
Percentage of S insoluble in acetic acid	.11	.13	.11	.11	.115		
Inoculated series, Medford loam:							
Percentage of N insoluble in acetic acid	.....	.....	.....	.....	2.20	17.6	11.0
Percentage of S insoluble in acetic acid	.....	.....	.....	.....	.125		
Uninoculated series, Medford loam:							
Percentage of N insoluble in acetic acid	.....	.....	.....	.....	1.21	20.0	.....
Percentage of S insoluble in acetic acid	.....	.....	.....	.....	.11		

To say that percentage of sulphur will not increase regardless of sulphate concentration in the nutrient media without increasing the available nitrogen would not be in accordance with data already obtained. It does appear, though, that when the lack of nitrogen is sufficient to lower the nitrogen content compared to the normal nitrogen content of the clover there is a tendency toward decreased sulphur assimilation. It is interesting to compare the rape plant with the clover in this respect.

The figures given in Table IV show that the clover cut before the blossoming stage not only contains a higher percentage of total nitrogen but also a higher percentage of nitrogen insoluble in acetic acid. As the percentage of nitrogen removed by this fraction varies, so also does the percentage of sulphur. There appears to be a definite relationship between the sulphur and nitrogen content in this insoluble portion, thus adding more significance to this fraction in regard to quality and perhaps representing the true protein of the clover hay. No difference in ash content caused by variation in fertilizer treatment was observed in the different pots. The ash content of the different crops did vary however, as is shown in Table IV.

EXPERIMENTAL WORK WITH THE RAPE PLANT

The Medford loam soil used in the first part of this work was used in this experiment. After the clover roots were removed the soil was returned to the pots and seeded to rape. Three gm. of sodium nitrate were added to those cultures which had received nitrate nitrogen in the clover experiment. The plants were harvested after a growing period of 50 days. At the end of this time there had been a cessation of growth, and the basal leaves dried up and fell off. The results appear in Table V.

TABLE V.—*Analytical results obtained with rape*

Treatment.	Number of plants.	Weight.	Total N.	N insoluble in acetic acid.	Total S.	Sulphate S.	Organic S.	Ash.
		Gm.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Control	[a] 7	12.0	1.93	0.90	0.35	0.16	0.19	13.6
	7	5.0	2.88	1.02	.39	.12	.27	19.3
$NaNO_3$	7	12 2	2.35	.93	.22	.03	.19	15.9
	[a] 7	10.1	4.14	.95	.16	.02	.14	18.0
$NaNO_3$ and Na_2SO_4	5	11.3	3.36	.71	1.46	1.09	.37	18.3
	[a] 6	14.0	2.72	.88	1.38	.97	.41	17.7
Na_2SO_4	7	2.4	2.57	.91	3.13	2.76	.37	22.0
	[a] 8	3.0	2.59	.....	2,41	2,05	.36	19.4
$NaNO_3$ and $CaSO_4$	[a] 6	14.45	2.30	.83	1.26	.83	.43	14.7
	6	16.35	2.27	.66	1.31	.93	.38	16.2
$CaSO_4$	[a] 7	2.4	2.69	.88	3.28	2.98	.30	21.9
	8	1.8	2.66	.....	2.70	2.37	.33	21.9

[a] Pots from the inoculated series.

The presence of added sulphates appeared to have retarded growth of the rape, as there is a greater dry-weight yield on the controls. With

the cultures receiving nitrate fertilizer the addition of sulphate sulphur apparently caused increased growth or had just the opposite effect. If the concentration of the sulphates was great enough to produce a toxic effect, the sodium nitrate may have counteracted this action.

The writer has observed just the opposite effect with clover seedlings growing on agar agar-mineral salt nutritive media. One gm. of sodium nitrate per liter had a noticeably toxic effect, while the same concentration of sodium sulphate produced no noticeably injurious effect. In the cultures containing both the same concentration of sodium nitrate and sodium sulphate there was an improvement in growth over the former sodium-nitrate cultures.

Application of nitrates produced very good yields on a comparative low sulphur assimilation by the plants. The question naturally arises whether the rape does not absorb sulphur, if present, far in excess of that required for carrying on the synthesis of its organic compounds. This appears so noticeable in comparing the figures in Table V. Of course it is realized that the optimum concentration of nutrients for plant nutrition has always been a problem. The acetic acid-insoluble nitrogen is higher in the rape grown on the soil receiving nitrate fertilizer only, compared with that in the rape which received both nitrate and sulphate fertilizer. There seems to be a tendency of the sulphates to decrease this form of nitrogen. Sulphate application increased the organic sulphur and total sulphur content of the rape, while at the same time the presence of these sulphate compounds retarded growth where no nitrates were added. The extremely high sulphate content is very obvious in these samples of rape. This may account for the high ash content. The percentage of ash in the samples of rape varies considerably, depending upon the fertilizer treatment and magnitude of growth. Such a variation did not occur with the clover.

SUMMARY

Sodium sulphate and calcium sulphate had a beneficial effect on nodule development and nitrogen assimilation of the red clover grown on previously sterilized soil. On a similar series which was artificially inoculated with *Bacillus radicicola* at the time of seeding, sulphates caused no increase in nodule development.

When a soil of high sulphur content was used, the nitrogen content in clover of the third and fourth crops was lower on the control pots than where either sulphur, calcium sulphate, or sodium sulphate was applied. As sulphate sulphur was present in all plants, the low nitrogen content could not be explained by a cessation in protein synthesis due to the absence of sulphates.

This again shows the relation of sulphates to nitrogen assimilation and the favorable influence of sulphates on the legume bacteria or on some other agency controlling nitrogen assimilation.

The ratio of nitrogen to sulphur in the portion of the clover plant insoluble in dilute acetic acid remains about the same, regardless of the stage in the development of the plant. This gives further support to the view that the nitrogen insoluble in acetic acid represents protein nitrogen. The total nitrogen and total nitrogen insoluble in acetic acid was higher in those plants cut before the blossoming stage.

With clover growing on sand cultures, it was possible, by reducing the available nitrate, not only to limit the growth and nitrogen content but also to decrease the sulphur assimilation. So, while sulphates apparently cause greater nitrogen assimilation through their beneficial effect on nodule development, the amount of sulphur taken up by the plant is limited by the total nitrogen absorbed.

The rape plant assimilated a large amount of sulphur, although the presence of sulphates reduced the yield compared to the control soil cultures. Sulphate plus nitrate caused increased yields compared with those secured when nitrate was added alone. There does not appear to be any direct relation between nitrogen and sulphur assimilation in the rape plant.

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SOYBEAN MOSAIC [1]

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In a small field of Hollybrook soybeans in West La Fayette a typical mosaic disease was found August 25, 1920. A rather low percentage of the plants were affected, and the disease was more or less confined to one quarter of the field adjacent to which were several rows of garden beans affected with mosaic to a considerable degree. In another larger field of soybeans in the same locality no mosaic was found. Leafhoppers were very prevalent on the soybeans. The impression was gained that the disease might have spread from the garden beans to the soybeans, but as yet no evidence to support such a theory has been obtained.

Clinton [2] found soybean mosaic in 1915 at Mount Carmel, Conn., and under the name of chlorosis or crinkling has given an excellent account of the leaf symptoms along with a good illustration. He found the disease on the varieties Medium Green, Wilson, Swan, Kentucky, Wing's Mikado, and Hollybrook, and states that the Hollybrook showed the most marked symptoms. He found the chlorosis without the crinkling on the varieties O'Kute, Ito San, and Manhattan. C. R. Orton [3] has reported the occurrence of mosaic in a field of Ito San soybeans at Girard, Pa., July 30, 1920.

SYMPTOMS

The mosaic symptoms on the soybeans were conspicuous and unmistakable, resembling those characteristic of mosaic diseases in general. Affected plants were stunted, and petioles and internodes were shortened to some extent. The leaflets were stunted, greatly misshapen, and puckered with dark-green puffy areas along the veins (Pl. 18, A, C, D, E). Between these puffy areas the leaf tissue was etiolated. Affected leaflets tended to be asymmetrical, twisted, and curled downward about the margins (Pl. 18, D, E). As in other mosaic diseases, the young, rapidly growing leaves showed the most severe effects, and in some cases whole leaflets or portions thereof were extremely stunted or killed outright by the disease (Pl. 18, B). The mosaic symptoms were readily distinguishable from a uniform crinkling of the leaflets which was rather common in this field and apparently attributable to insect injury.

The pods on mosaic plants were stunted and flattened, less pubescent, and more acutely curved than those on normal plants (Pl. 19, C, D).

[1] Contribution from the Botanical Department of Purdue University Agricultural Experiment Station, La Fayette, Ind.

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[3] FROMME, F. D. DISEASES OF CEREAL AND FORAGE CROPS IN THE UNITED STATES IN 1920. *In* U. S. Dept. Agr. Bur. Plant Indus. Plant Disease Bul., Sup. 15, p. 173. 1921. Mimeographed.

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Those borne at the upper nodes were more severely affected. The yield of seed was very materially reduced (Pl. 19, A, B), since a considerable proportion of the pods contained no germinable seeds and the remainder as a rule not more than one or two seeds (Pl. 19, D). Even the germinable seeds were in general undersized.

Observations made a month later showed that the mosaic plants were remaining green longer than the normal plants, so the disease evidently delayed maturity.

FIELD INOCULATIONS

In another field of soybeans in which no mosaic was present inoculations were made August 27 by rubbing the young internodes with cotton soaked in the juice from crushed mosaic soybean leaves and then wounding these internodes with a needle. One hundred and fourteen plants were thus inoculated, but no mosaic developed. Fifty-two plants were similarly inoculated, except that the juice of leaves from mosaic garden beans was used as inoculum, and none developed the disease. Forty-six garden bean plants were also inoculated in a similar manner with the virus from soybean mosaic, and none developed mosaic.

SEED TRANSMISSION

To determine whether or not the disease was seed-borne, a quantity of seed was saved from mosaic and healthy plants early in October for subsequent tests in the greenhouse. On October 25, 150 seeds from mosaic plants were planted in 25 pots of sterilized soil, 6 in each pot. By December 15, 124 plants had come up, and 18 showed unmistakable mosaic symptoms. None of the 148 controls grown from seed from normal plants showed mosaic.

In a second trial about 180 seeds from mosaic plants were planted December 9 in 59 pots of sterilized soil. February 3, 1921, 11 out of the 106 plants which were up showed mosaic. None of the 38 controls grown from seed from normal plants showed the disease. As a result of these two tests it is evident that about 13 per cent of the seedlings from seed produced on mosaic plants developed the disease.

The mosaic seedlings were spindling (Pl. 18, F, G), and the first pair of true leaves were characterized by downward, longitudinal curling or rolling, a crinkling, and a faint etiolation or mottling. These leaves turned yellow prematurely. The leaves subsequently formed were greatly stunted and showed the mottling and crinkling more conspicuously than the first leaves.

GREENHOUSE INOCULATIONS

From these mosaic seedlings the disease was transmitted to healthy soybean seedlings. Several methods of inoculation proved successful. A number of inoculations made early in January yielded only negative results, but later better success was obtained.

On January 26, twenty-five plants were inoculated by pricking with a needle at the nodes and rubbing the wounded areas with cotton soaked in the juice from crushed mosaic leaves. Eight plants used as controls were similarly treated, except that sterile water was substituted for the mosaic virus. Because of the unfavorable greenhouse conditions the plants made slow growth during the winter, so that the mosaic symptoms were very slow in developing. On March 5 two plants showed mosaic mottling on the young leaves. On March 25 two more showed mosaic, and on April 7 seven out of the 25 plants had developed the disease. The controls developed no mosaic.

A number of inoculations were made March 2. In one series crushed mosaic tissue was inserted into slits made with a scalpel near the growing points and on the petioles. On March 15 two of the seven plants thus inoculated showed mosaic symptoms on the young leaves, and on April 7 five had developed mosaic.

In a second series of inoculations made the same date by cutting off one leaf at each node and smearing these wounded surfaces with crushed mosaic tissue, three out of eight plants showed mosaic symptoms on the new leaves March 15, or 13 days after inoculation, and on April 7 six plants had developed mosaic.

In a third series five plants were inoculated by a combination of the two methods above described. Thirteen days later three showed mosaic mottling, and by April 7, or 37 days after inoculation, four had developed the disease.

In a fourth series, five plants were inoculated by rubbing the under surfaces of the leaves with slightly crushed mosaic leaves forcibly enough to cause slight abrasions. On March 15, four of these plants showed the disease, and on April 7 all showed typical mosaic.

None of the five control plants inoculated by one or the other of these methods without the application of mosaic tissue developed mosaic. At no time was there any spread of the disease in the greenhouse.

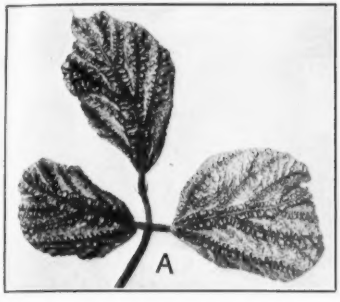
In these inoculations the symptoms became evident only on the young leaflets. These in some cases developed distinct mottling, and in other cases they exhibited a slight degree of etiolation and the characteristic downward, longitudinal rolling. The incubation period under the conditions of this test was 13 days.

Preliminary cross inoculations to garden beans and cowpeas have given negative results. Further tests are being made.

Soybeans, therefore, are subject to a destructive mosaic disease which greatly reduces the yield of affected plants. The disease is transmissible from plant to plant and also is seed-borne.

PLATE 18

A.—Typical mosaic leaf showing darker green puffy areas along the veins.
B.—Mosaic leaf showing extreme stunting of terminal leaflet.
C.—Normal leaflet.
D.—Mosaic leaflet showing longitudinal rolling.
E.—Typical mosaic leaflet.
F.—Mosaic seedlings from seed from a mosaic plant, showing stunting of the plant and longitudinal rolling of first leaves.
C.—Normal seedlings from seed from a mosaic plant.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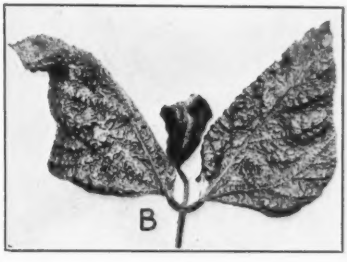
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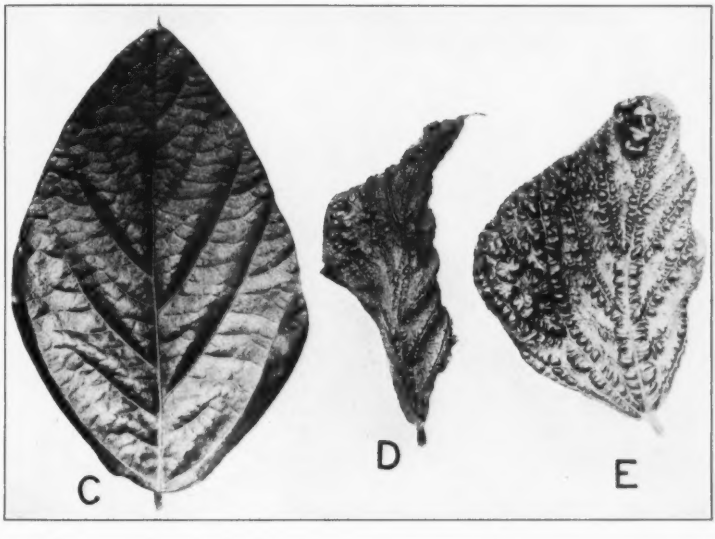
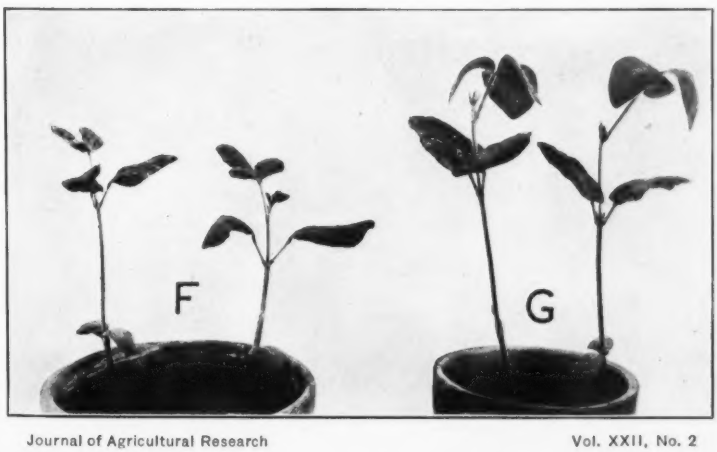

C
D
E


F
G

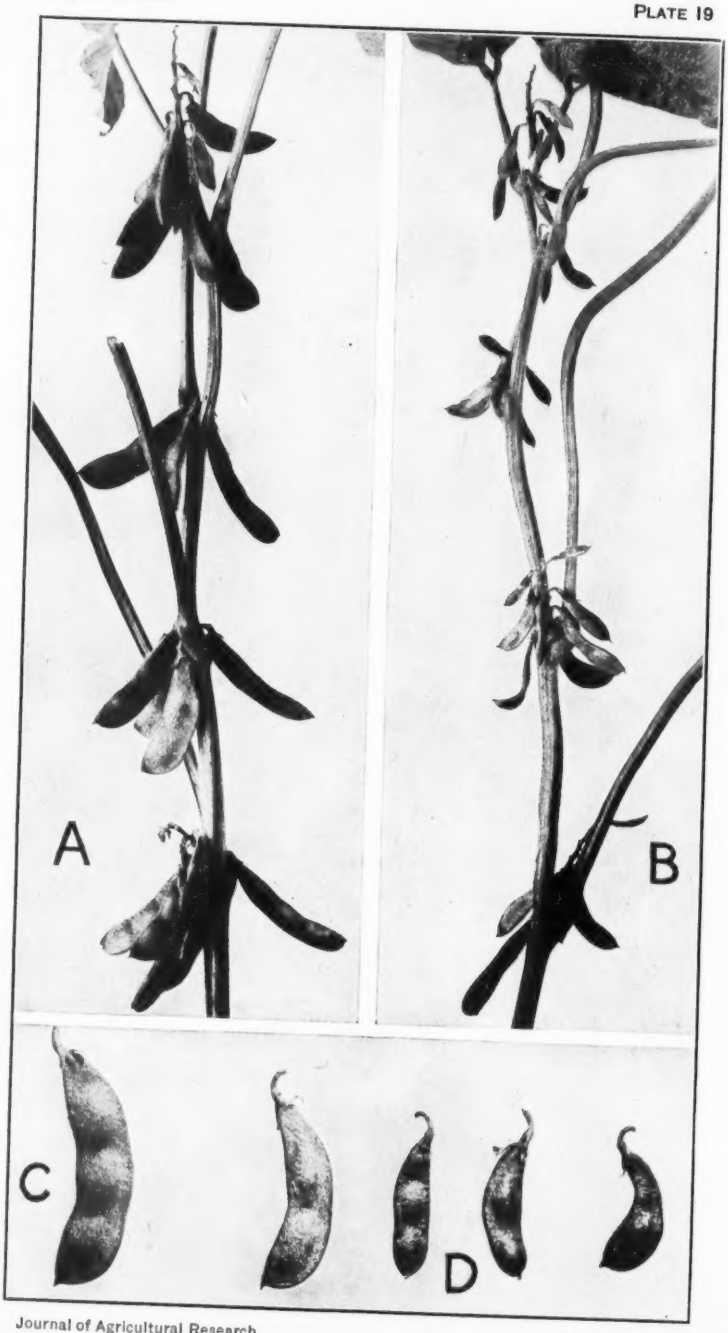

A
B
C
D

PLATE 19

A.—Upper nodes of a normal plant, showing yield of pods.
B.—Upper nodes of a mosaic plant, showing effect of the disease on the yield.
C.—Normal pod.
D.—Type of pods produced by a mosaic plant.

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